

# Tyrosine dose does not influence exercise performance in the heat

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## Abstract

**Purpose:** An acute dose of tyrosine, 1 hour before exercise has been associated with improved exercise capacity in a warm condition although the same dose has failed to demonstrate any benefit on exercise performance at the same environmental temperature. The present study sought to determine whether administering different doses of tyrosine across trials would demonstrate whether an optimal dose of tyrosine exists for improving exercise performance in the heat.

**Method:** Following familiarisation, 8 healthy male volunteers who were unacclimated to exercise in the heat, performed 4 experimental trials at 30°C/60% relative humidity, consuming one of four drinks (placebo, 150 mg kg body mass<sup>-1</sup>, 300 mg kg body mass<sup>-1</sup> or 400 mg kg body mass<sup>-1</sup>) in a randomised crossover design, separated by at least 7 days. In the hour prior to exercise, participants consumed one of the four experimental drinks before exercising for 1 hour at 10% Δ (129 ± 17 W) and then completing a simulated time trial as quickly as possible which required the completion of an individualised target work quantity (326 ± 37 kJ).

**Results:** Time trial time ( $P = 0.553$ ) and time trial power output ( $P = 0.281$ ) remained similar across all trials. The plasma ratio of tyrosine:  $\sum$ LNAA was similar at rest between all trials ( $P = 0.657$ ) but increased significantly from rest in all tyrosine conditions ( $P < 0.01$ ;). The tyrosine ratio increased 4.8 fold from baseline in LOW, 7.3 fold in MED and 7.3 fold in HIGH. Core temperature ( $P = 0.326$ ), skin temperature ( $P = 0.127$ ), heart rate ( $P > 0.05$ ), Ratings of perceived exertion ( $P > 0.05$ ) and thermal sensation ( $P > 0.05$ ) also remained similar across all trials.

**Conclusion:** The data demonstrates that acute administration of a range of tyrosine doses has no beneficial effect on exercise performance in the heat despite marked increases in plasma tyrosine concentration.

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## Abbreviations

ANOVA: Analysis of Variance  
BCAA: Branched chain amino acids  
DMTS: Delayed matching to sample task  
EEG: Electroencephalogram  
g: grams  
GCMS: Gas Chromatography Mass Spectroscopy  
Il: Illinois  
kg: Kilogram  
km: Kilometre  
LNAA: Large neutral amino acids  
MED: Medium  
mg: Milligram  
Mins: Minutes  
mL: Millilitres  
PLA: Placebo  
PO: Power Output  
PTT: Post time trial  
RPE: Rating of Perceived exertion  
SD: Standard Deviation  
TT: Time Trial  
UK: United Kingdom  
USA: United States of America  
 $\dot{V}CO_2$ : Carbon dioxide production  
 $\dot{V}O_2$  max: Maximal oxygen uptake  
 $\dot{V}O_{2\text{ peak}}$ : Peak oxygen uptake  
W: Watts  
5-HT: Serotonin

## **Literature Review**

Physical exercise in all mammals results in an increase in internal heat production and core temperature (Wednt, Loon and Marken Lichtenbelt, 2007). Humans have evolved a number of physiological mechanisms, such as vasodilation, vasoconstriction and sweating, in order to not only survive in warm temperatures, but to perform tasks effectively in high temperatures for prolonged periods of time. Despite this, it is well documented that prolonged exercise in the heat is significantly impaired in comparison to exercise in cooler conditions (Galloway and Maughan, 1997; Febbraio, Snow, Stathis, Hargreaves and Corey, 1994; Tatterson, Hahn, Martin and Febbraio, 2000). There is still much debate as to the precise mechanisms which cause fatigue during exercise, defined as “an increase in perceived effort required to exert a desired force or power output and the subsequent inability to produce that power output” (Davis and Bailey, 1997). Exercise capacity is defined by Goldstein (1990) as the maximum amount of physical exertion that can be sustained at a given work intensity, while exercise performance is defined by Coyle (1999) as the amount of power or velocity that can be maintained over a set period of time. Central fatigue (fatigue within the central nervous system) or peripheral fatigue (fatigue outwith of the central nervous system), or a combination of both processes, have all been argued to be the primary influence on exhaustion during exercise.

When the human body encounters increased heat stress, developed through metabolic heat, external heat or a combination of both, it responds in a number of ways to maintain thermal homeostasis and assist in delaying fatigue. These responses to heat stress are co-ordinated by the hypothalamus, an area of the brain responsible for maintaining thermoregulation. The hypothalamus plays a vital role in controlling body temperature by coordinating thermal



information from all areas of the body and directing the efferent signals to the appropriate heat production and heat conservation systems in mammals (Cooper, 2002). Changes in body temperature are detected by thermosensitive neurons, which have the ability to monitor the temperature of blood flowing into the brain and send feedback to the hypothalamus; effectively detecting changes in core temperature (Wednt, Loon and Marken, 2007). When challenged with an increasing body temperature, the hypothalamus will respond by initiating the processes of vasodilation and sweating. These are considered the primary peripheral mechanisms by which humans lose heat in high ambient temperatures or during exercise, in order to maintain thermal homeostasis. The combination of exercise and high ambient temperatures poses a particularly challenging situation for human thermal physiology.

Physical exercise results in an increased internal temperature and so therefore there is a simultaneous demand for blood from both skeletal muscle and the skin. This can pose a particular circulatory challenge when exercise is performed in the heat. The need for an increased blood flow to the skin, especially at high core temperatures, is to facilitate heat exchange with the environment through evaporative sweat loss. The evaporation of sweat from the skin decreases skin temperature and subsequently lowers the temperature of the blood in the dilated vessels near the body's surface before it returns to the core, slowing the increase in core temperature (Shirreffs, Aragon-Vargas, Chamorro, Maughan, Serratos and Zachwieja, 2005); Charkoudian, 2003). The effectiveness of the vasodilatory process is highlighted by the suggestion in the aforementioned study that skin blood flow can increase from  $0.25 \text{ L}\cdot\text{min}^{-1}$  at rest to  $8.00 \text{ L}\cdot\text{min}^{-1}$  during physical activity in warm environments. If the body is unable to match heat gain with heat dissipation, then temperature will begin to increase, which will eventually begin to impair exercise performance (Casa, 1999). A study by Gonzalez-Alonso *et al.*, (1999) examined the influence of body temperature on fatigue during prolonged exercise.

The study found peripheral factors such as increased heart rate and reduced stroke volume contributed significantly to a shorter time to exhaustion when participants reached critical hyperthermia (40.1 – 40.2 °C).

It has been suggested that the ability of humans to exercise is limited by peripheral mechanisms such as metabolic and cardio-respiratory capacity (Kayser, 2003). Examples of this include diminishing substrate availability in working muscle, circulatory factors such as an attainment of peak oxygen uptake ( $\dot{V}O_2$  peak) and an increasing concentration of blood lactate (Kayser, 2003). Other studies however, have suggested that fatigue in warm conditions exhibits a large central element. Nielsen, Hyldig, Bidstrup, Gonzalez-Alonso and Christoffersen (2001) observed alterations in the electrical activity of the brain's frontal area measured via electrodes attached to the scalp, when subjects became hyperthermic during exercise in the heat. The study found that electrical activity in the prefrontal cortex increased initially from rest to the start of exercise before the high frequency  $\beta$  band began to gradually decline. These changes are associated with reduced arousal. This occurred at the same time as a progressive increase in oesophageal temperature which continued to rise until exhaustion. The authors summarised that these changes indicated hyperthermia associated fatigue. In a separate study using a similar method to measure prefrontal cortex electrical activity, Nybo and Nielsen (2001) observed changes to cerebral electroencephalography (EEG) which was correlated with increases in ratings of perceived exertion, when exercising in the heat (40°C). Although there is a large body of evidence that demonstrates that there are various factors that can result in fatigue during exercise in the heat, it is stated by Kayser (2003) that all voluntary exercise starts and ends in the brain.

The interaction between the brain neurotransmitters serotonin (5-hydroxytryptamine) and dopamine has been suggested to have a regulative role in the development of fatigue (Meeusen,

Watson, Hasegawa, Roelands and Piacentini, 2006). Dopamine, serotonin and noradrenaline are the principle neurotransmitters that regulate a number of functions within the central nervous system, which include motor control, cognition, emotion, memory processing, stress responses and endocrine regulation (Kobayashi, 2001). The original central fatigue hypothesis proposed by Newsholme, Acworth and Blomstrand (1987), was based solely on the action of serotonin and suggested that increased brain serotonergic activity may augment loss of drive and lethargy during prolonged exercise resulting in a reduction in motor unit recruitment and exercise performance. Prolonged exercise has been shown to increase free fatty acids in the blood. This results in an increase in blood free-tryptophan concentration, serotonin's precursor, as free fatty acids displace tryptophan from its binding sites on albumin molecules in the bloodstream (Davis, 1995). However evidence for the original fatigue hypothesis is mixed with pharmacological manipulation of serotonin proved successful for example by Blomstrand, Hassmen, Ek, Ekblom and Newsholme (1997), but unsuccessful by Varnier *et al.*, (1994). Conversely dopamine has been associated with increased motivation, arousal, memory, reward mechanisms and increased attention (Meeusen *et al.*, 2006). Davis and Bailey (1997) proposed a revised central fatigue hypothesis that a high ratio of brain dopamine: serotonin will assist in prolonging exercise and delaying fatigue, whereas when dopaminergic activity is reduced fatigue is precipitated by a loss of coordination and motivation. Burgess, Davis, Borg and Buggy (1991) demonstrated that rats were able to exercise for a longer period of time when they were able to self-stimulate the ventral tegmental, an area of the brain high in dopaminergic pathways. As a potent dopamine releaser, studies have also demonstrated improved exercise performance with amphetamine administration in humans (Chandler and Blair, 1980). The importance of high dopaminergic activity led various authors to pharmacologically manipulate catecholamine activity to assess the effect on exercise performance. Interestingly, it was found by Watson, Hasegawa, Roelands, Piacentini, Loooverie and Meeusen (2005) that exercise in the heat was enhanced by acute administration of bupropion,

a dual dopamine/noradrenaline reuptake inhibitor, in humans, however there was no effect found when exercise took place within a temperate climate. The authors suggested a possible increase in the risk of heat illness due to bupropion's ability to dampen or override inhibitory signals arising from the CNS to cease exercise due to hyperthermia and enable an individual to continue to maintain a high power output (Watson *et al.*, 2005). Hasegawa, Piacentini, Sarre, Michotte, Ishiwata and Meeusen (2008) demonstrated that exercise performance in the heat was improved in rats with bupropion administration via acute injection. Samples gathered directly from the preoptic area and anterior hypothalamus in the brain, using microdialysis, showed increased extracellular concentrations of dopamine and noradrenaline during exercise in the heat, especially with bupropion administration compared to the control group. This is supported in an earlier study by Piacentini, Clinkers, Meeusen, Sarre, Ebinger and Michotte (2003) which found bupropion administration increased brain dopamine concentration in rats when measured via microdialysis. A study by Bridge, Weller, Rayson and Jones (2003) demonstrated that high dopaminergic activity in the brain can delay fatigue and improve exercise ability in the heat. Bridge *et al.*, (2003) gave participants either buspirone (dual 5-HT<sub>1a</sub> receptor agonist and DA D<sub>2</sub> receptor antagonist) or pindolol (5-HT<sub>1a</sub> receptor antagonist) before participants exercised in the heat until exhaustion. The provision of both buspirone and pindolol was used to indirectly assess the dopaminergic and serotonergic components of prolactin release. Blood prolactin concentration is often used as an indicator of dopamine and serotonin activity as the release of prolactin from the pituitary, and the subsequent presence of prolactin in the blood, is stimulated by serotonin but inhibited by dopamine. A positive relationship was found between exercise tolerance and the non-serotonergic component of prolactin response to buspirone. The authors concluded that improved exercise tolerance was most likely due to increased activity of dopamine in the hypothalamus. This is supported by Cordery, James, Peirce, Maughan and Watson (2016) which demonstrated increases in prolactin concentration following exercise were

attenuated with administration of the dopamine precursor L-DOPA. The conclusions drawn from these studies suggest that prolonged exercise in the heat may impose a specific demand on central dopamine systems which is not present in cooler environments and that pharmacological manipulation can influence this in both rats and humans.

Based on the evidence that dopamine plays an important role in exercise tolerance in the heat, Tumilty, Davison, Beckmann and Thatcher (2011) suggested that any substance that has the ability to increase dopamine availability should prolong exercise in the heat. The study demonstrated that an acute dose of a nutritional dopamine precursor, tyrosine, was associated with an improvement in exercise capacity in the heat. Watson, Enever, Page, Stockwell and Maughan (2012), however, found that tyrosine had no effect on exercise capacity in the heat when providing participants with a similar dose and using a similar exercise protocol. Other studies by Tumilty, Davison, Beckmann and Thatcher (2014) and Coull, Christmas, Watson, Horsfall and Taylor (2015) also both found tyrosine to have no effect on exercise performance in the heat. It is possible that the stress of the exercise protocol was insufficient to increase catecholamine metabolism or that the dose of tyrosine used in these studies was insufficient to maintain catecholamine metabolism due to depletion of precursor availability. Evidently, the use of nutritional precursors in an attempt to influence central neurotransmitters has so far produced mixed results during exercise in the heat, and therefore further investigation is necessary. Despite mixed results in exercise trials, the effects of such precursors have shown promising results on cognitive function, which is discussed in more detail below.

### **The Blood-Brain Barrier**

Tyrosine, as well as other large neutral amino acids (LNAA), must cross the blood brain barrier in order to exert effects on catecholamine metabolism in the brain. The blood-brain barrier is a

semi-permeable barrier which impedes influx of most compounds from the blood to the brain. There are three cellular elements of the brain's microvasculature which compose the blood brain barrier – endothelial cells, astrocyte end feet and pericytes (Ballabh, Braun and Nedergaard, 2004). Pachter, de Vries and Fabry (2003) state that the blood-brain barrier provides both anatomical and physiological protection for the central nervous system, strictly regulating the entry of many substances and blood borne cells into the nervous tissue. Entry of amino acids and other metabolites into the brain, which circulate within the blood and compete for uptake, takes place through the blood brain barrier. Tyrosine, tryptophan and phenylalanine (tyrosine precursor) all share a LAT-1 transporter with other LNAA (Oldendorf and Szabo, 1976). The competition for uptake at the blood-brain barrier is highlighted in a study by Fernstrom and Wurtman (1972). This examined the effect of whether feeding protein in varying amounts would increase tryptophan uptake into the brain. Rats were provided with either a meal containing an amino acid mixture containing LNAA or a meal minus tryptophan's suspected transport competitors, including tyrosine, phenylalanine, leucine, isoleucine, valine. It was reported that the rats who consumed the meal which lacked the suspected transport competitors displayed significant rises in brain tryptophan and serotonin synthesis. Despite large rises in plasma tryptophan concentration following the amino acid mixture meal, the increase was offset at the transport sites by a comparable and proportional increase in the plasma levels of tryptophan's competitors (Fernstrom and Wurtman, 1972). Importantly, the meal lacking LNAA increased the blood ratio of tryptophan: LNAA. This is important as the blood ratio of an individual amino acid: amino acids competing for brain uptake is a key determinant of transport into the brain. Therefore, brain uptake of an individual amino acid can be increased by acutely increasing the blood concentration of that amino acid, or by reducing the concentration of amino acids competing for uptake (in both cases this would increase the blood ratio: competing amino acids). This is supported by experimental data in rats. Fernstrom and Faller (1978) demonstrated that

the concentration of branched chain amino acids (BCAA) (valine, leucine and isoleucine) changes rapidly in the brain following the ingestion of a meal. Ingestion of a meal with 0% protein was shown to significantly increase levels of brain tryptophan, in parallel with a rise in serum tryptophan, compared to a meal containing either 18% or 40% protein. Despite significant increases in serum tryptophan following ingestion of the 18% and 40% protein rich meals, brain tryptophan concentration did not increase. This was due to proportionally similar increases in blood concentrations of competing LNAA, with little change in the ratio of blood tryptophan: LNAA (Fernstrom and Faller, 1978). Mauron and Wurtman (1982) demonstrated in rats that when a  $3 \text{ g} \cdot \text{kg}^{-1}$  dose of glucose was administered alongside tyrosine, the ratio of serum tyrosine: LNAA and brain tyrosine concentration increased. The increase in brain tyrosine concentration can be attributed not only to the additional tyrosine but also to the glucose mediated insulin release which works to lower blood LNAA concentrations via uptake in peripheral tissues (Mauron and Wurtman, 1982). When the concentration of a particular amino acid increases in the brain, there may be a subsequent increase in that amino acid's neurotransmitter under suitably demanding conditions. Brady, Brown and Thurmond (1980) displayed significant increases in both brain tyrosine and dopamine in mice following tyrosine administration. Similarly, administration of tyrosine has been shown to increase dopamine release in rats in a study by Acworth, During and Wurtman (1988). This study demonstrated that tyrosine caused a dose-related increase in brain extracellular fluid dopamine levels, measured using brain microdialysis.

### **Monoamine and Catecholamine Synthesis**

Tryptophan and tyrosine are considered to be unique among amino acids in being precursors to brain neurotransmitters (Fernstrom, 2013). Variations in the brain concentrations of tryptophan and tyrosine affect the synthesis and release of their respective neurotransmitters. Increasing

concentrations of brain tryptophan generally result in increased brain serotonin synthesis. Increased brain tyrosine concentration may result in greater synthesis of the brain neurotransmitters dopamine, norepinephrine (and epinephrine, less so) but only under suitably demanding conditions which increase the firing rates of catecholamine neurons. (Fernstrom, 2013). Serotonin is synthesised from tryptophan using a two-step process, beginning with hydroxylation. Serotonin is produced by decarboxylation of 5 – hydroxytryptamine before serotonin is released from nerve terminals to interact with serotonin receptors on adjacent neurons (Fernstrom, 1983). Rapid transmission of the neurotransmitter then occurs across the synaptic cleft via a serotonin transporter before serotonin is either stored or metabolized (Fernstrom, 1983). Tyrosine and phenylalanine are considered to be the major precursors for the synthesis of dopamine and norepinephrine and can be found in abundance in natural dietary proteins (Elsworth and Roth, 1997). Blood-borne tyrosine is taken up into the brain where it is then transported from brain extracellular fluid to dopaminergic neurons by amino acid transporters (Elsworth and Roth, 1997). The primary stage in the synthesis of these monoamines requires the addition of a hydroxyl group to the aromatic ring of tyrosine or the indole nucleus of tryptophan (Gibson and Wurtman, 1976). This process is catalysed by tyrosine hydroxylase and tryptophan hydroxylase, respectively, resulting in the creation of the hydroxylated products dihydroxyphenylalanine (DOPA) and 5-hydroxylated tryptophan. These are then both decarboxylated in separate neuron populations to form dopamine and serotonin (Gibson and Wurtman, 1976). To form noradrenaline, dopamine is  $\beta$ -hydroxylated by dopamine  $\beta$ -hydroxylase. Noradrenaline is a key modulator of stress response in mammals and therefore, tyrosine, as a precursor, should prevent norepinephrine depletion and therefore maintain its synthesis during rapid neuronal firing. In order to produce adrenaline, noradrenaline is methylated by phenylethanolamine-N-methyl-transferase (Cooper, 2003).



Evidence from previous studies demonstrates that tyrosine supplementation is more effective in increasing the synthesis and release of catecholamines if catecholamine neurons fire at an increased rate. This is linked to the underlying biochemical mechanisms controlling brain catecholamine synthesis. At basal levels, tyrosine hydroxylase tightly controls dopamine synthesis. Increased dopaminergic neuronal firing as a result of stress exposure in rats, such as cold stress, tail shock, heat stress or forced swim stress, may deplete brain dopamine as a result of depleted precursor availability in some neuronal populations (Fernstrom, 1983). Additional tyrosine availability in such conditions assists in maintaining catecholamine turnover and may work to maintain or prevent the decline performance in demanding conditions. For example, additional tyrosine can increase dopamine synthesis and release depending on the firing rates of nigrostriatal dopaminergic neurons in rats (Melamed, Hefti and Wurtman, 1980). Mice exposed to cold swim stress demonstrated decreased levels of dopamine although this decline was alleviated by tyrosine administration (Brady *et al.*, 1980) suggesting tyrosine maintained catecholamine turnover during stress exposure. During, Acworth and Wurtman (1988) demonstrated an enhanced effect of tyrosine on dopamine release following partial lesioning of nigrostriatal neurons, but only in surviving neurons. Therefore, additional tyrosine availability in such conditions assists in maintaining catecholamine turnover. Based on this, it is plausible to suggest that precursor availability is a major limiting factor in catecholamine production, but only under very demanding conditions which sufficiently upregulate brain catecholamine function (Fernstrom, 1983). In conditions which do not suitably stress dopaminergic neurons, any additional tyrosine is likely to be affected by end-product inhibition of tyrosine hydroxylase. This is demonstrated by Badaway and Williams (1982) who found that when rats were provided with a 25 mg·kg<sup>-1</sup> tyrosine they displayed increases in catecholamine synthesis but when administered with > 50 mg·kg<sup>-1</sup> tyrosine, there was no such increase. Given that the rats were

unstressed (i.e. no specific experimental stress protocol was present) it is perhaps unsurprising that the larger dose of tyrosine failed to increase catecholamine synthesis.

### **Catecholamines and Prolonged Exercise in the Heat**

The original central fatigue hypothesis was based upon the actions of serotonin, with the assumption that the increased presence of serotonin during exercise will augment loss of drive, lethargy and result in a decreased level of performance. This original hypothesis has since been developed and now encompasses the synthesis and metabolism of catecholamines, such as dopamine and noradrenaline to form the basis of the revised central fatigue hypothesis (Meussen *et al.*, 2006). Evidence also suggests that dopamine may be implicated in thermoregulation during exercise, in rat (Hasegawa, Piacentini, Sarre, Michotte, Ishiwata and Meeusen, 2008) and human studies (Watson *et al.*, 2005).

Hasegawa *et al.*, (2008) examined how brain catecholamines influenced the development of fatigue in rats during exercise in the heat. The aim of the study was to identify if there was any effect on exercise performance following an acute injection of bupropion. The study also monitored the effects of bupropion on thermoregulation and neurotransmitter activity in the anterior hypothalamus and preoptic area in the rat during exercise via a microdialysis probe. The rats exercised to exhaustion in three trials, in 30°C with either bupropion or saline as placebo and at 18°C. The results demonstrated time to exhaustion was significantly higher in the heat following intake of bupropion despite significantly higher core and brain temperatures compared to exercise in the heat with placebo. Extracellular levels of dopamine and noradrenaline increased during exercise, but were significantly higher in the group who were administered the

bupropion, suggesting that an increased level of dopamine and noradrenaline in the preoptic area and anterior hypothalamus may be responsible for an increase in exercise performance.

A study by Watson *et al.*, (2005) reported that acute administration of bupropion, a dual dopamine/noradrenaline reuptake inhibitor, resulted in improved exercise performance in the heat but not in a temperate condition, suggesting there may be a specific demand for dopamine during exercise in the heat. Here it was proposed that bupropion acted on dopamine neurotransmission to maintain motivation and arousal and allowed subjects to overcome fatigue sensation. Bridge *et al.*, (2003) also found time to exhaustion was positively influenced by dopaminergic pathways when participants were administered either buspirone or pindolol, the provision of which was used to indirectly assess the dopaminergic and serotonergic components of prolactin release. The study found a correlation between time to exhaustion and the non-serotonergic component of prolactin response to buspirone administration. Similarly to Watson *et al.*, (2005), work by Roelands *et al.*, (2008a) examined how a dopamine reuptake inhibitor affected exercise performance and thermoregulation during exercise in both hot and temperate conditions. Eight male subjects consumed a placebo or a dose of ritalin, an amphetamine-like agent containing methylphenidate, prior to exercise in either a warm condition (30°C) or a temperate condition (18°C). Ritalin has a high affinity for the dopamine transporter, which inhibits the reuptake of neuronal dopamine within the brain. Participants were required to cycle for sixty minutes at 55% W<sub>max</sub> (55% of the total watts achieved during a preliminary maximal exercise test) which was immediately followed by a time trial, equivalent to the amount of work equal to 30 minutes of exercise at 75% W<sub>max</sub>, to assess exercise performance. It was found that participants who ingested ritalin prior to exercise in the heat performed the time trial 16% faster compared to the placebo group. However, exercise in the temperate climate was unaffected by ritalin ingestion, demonstrating once more that brain dopamine availability positively influenced

exercise tolerance in the heat. Another study by Roelands *et al.*, (2008b) (using the same exercise protocol described above) demonstrated that norepinephrine reuptake inhibition via oral administration resulted in decreased exercise performance in temperate and warm conditions. This was accompanied by increased heart rate and increased subjective feelings of cold with norepinephrine reuptake inhibition, which may have negatively affected exercise performance. Norepinephrine's association with serotonin synthesis suggests that serotonin neurotransmission was potentially increased with norepinephrine reuptake inhibition. Norepinephrine neurones modulate the serotonin system via excitatory 1-adrenoceptors (Roelands *et al.*, 2008b). These studies provide persuasive evidence that increased dopamine availability through administration of reuptake inhibitors and amphetamine can improve exercise performance in the heat. It is also evident that there may be specific demand for dopamine in the heat which is not present during exercise in temperate conditions.

### **Nutritional Manipulation of Central Monoamines in Animals and Humans**

**(See summary table 1)**

Serotonin's association with fatigue has led a number of studies to examine the effects of nutritional and pharmacological manipulation of serotonin in both animals and humans through a variety of means. Soares, Coimbra and Marubayashi (2007) found that when tryptophan was injected directly into the lateral cerebral ventricle area of the brain in rats, exercise fatigue occurred more rapidly compared to when a saline solution was administered. Other studies in rats generally confirm that pharmacological manipulation of brain serotonin impairs performance (Bailey, Davis and Ahlborn 1992; Bailey, Davis and Ahlborn, 1993), although results are somewhat mixed in humans (Pannier, Bouckart and Lefebvre, 1995; Piacentini Meeusen, Buyse, De Schutter and De Meirleir, 2002a). Investigations have administered branched chain amino acids in order to assess the effects on brain serotonin and exercise performance, many of which

have produced mixed findings. In one study, Choi, DiSilvio, Fernstrom and Fernstrom (2013) examined whether oral administration of BCAA to rats would reduce both serotonin and catecholamine synthesis through decreases in brain tryptophan and tyrosine. The study demonstrated that BCAA administration did reduce serotonin and catecholamine synthesis 1 hour after BCAA intake. However, earlier work by Verger, Aymard, Cynobert, Anton and Luigi (1994) demonstrates that when rats were administered BCAAs, they fatigued quicker during prolonged treadmill running compared when either water or glucose was provided, demonstrating that results in rats are mixed. Blomstrand *et al.*, (1997) examined whether the acute intake of BCAA in humans would prevent the increase in free tryptophan, reduce brain uptake of tryptophan and thus delay fatigue. Seven male participants performed two bouts of exhaustive exercise on a cycle ergometer after performing a bout of exercise the previous day at 70%  $\dot{V}O_{2peak}$ . This was followed by 20 minutes of maximal exercise designed to deplete muscle glycogen stores. Participants received either BCAA or placebo during each trial. They found that ingestion of a BCAA solution was associated with an improved mental performance and a reduction in rating of perceived exertion (RPE) during the bout of prolonged exercise. Furthermore, it was reported that the ratio of the plasma concentration of tryptophan: BCAA was significantly increased in the placebo trial in comparison to the BCAA trial. This demonstrated a link between the reduced ratio of blood tryptophan to BCAA, an improved mental performance and a reduction in the subjective perception of work. There are, however, other studies which have failed to find any performance enhancing effect of BCAA administration during exercise while studies examining tryptophan administration have produced mixed results. Varnier *et al.*, (1994) found no differences in exercise performance when participants were provided with either 20 g of BCAA or saline, 70 minutes prior to a graded incremental exercise bout to exhaustion. Furthermore, Davis, Alderson and Welsh (2000) found BCAAs to have no effect on exercise capacity, RPE or cardiovascular and metabolic function during exercise to fatigue at 70%  $\dot{V}O_2$

max. The weight of evidence from the literature suggests that administration of BCAA generally does not enhance exercise performance or delay fatigue in humans, despite a sound physiological basis for the intervention (Davis, Alderson and Welsh, 2000).

A number of studies have also found that other forms of pharmacological manipulation of brain serotonin have had no effect on exercise performance in humans. Pannier *et al.*, (1995) found that, despite administration of the antiserotonin agent pizotifen, a 5-HT receptor antagonist, exercise performance did not improve. They found that performance level was lower when pizotifen was administered in comparison to placebo. Studies by Piacentini *et al.*, (2002a) and Piacentini, Meeusen, Buyse, De Schutter, Kempenaers, Van Nijvel, and De Meirleir (2002b) both found no effect on performance when venlafaxine, a serotonin/noradrenaline reuptake inhibitor and reboxetine, a noradrenergic reuptake inhibitor, respectively, were administered to 7 well trained male cyclists. Despite finding no effect on exercise performance, both studies did discover increased neurotransmitter activity in response to drug administration demonstrating a possible central effect. Segura and Ventura (1988) examined how supplementing tryptophan would affect exercise performance. Following two trials, where participants exercised at a workload of 80%  $\dot{V}O_2$  max with ingestion of either tryptophan or a placebo, it was found that exercise time was 49.4% greater with tryptophan ingestion in comparison to placebo. This is somewhat surprising given the proposed effects of serotonin on the brain and the influence on fatigue processes. This also highlights the mixed results in studies which have manipulated serotonin levels using various forms of dietary means. Stensrud, Ingier, Holm and Stromme (1992) conducted a similar study, and provided participants with either tryptophan or placebo to ascertain whether the results would be replicated. Following two trials to exhaustion, with either the tryptophan supplement or a placebo, it was found that there was no significant difference between the two trials. This emphasised that supplementation of tryptophan did not enhance

running performance, raising questions about the results produced by the original study by Segura and Ventura (1992). This led these authors, and others (Davis *et al.*, 1992) to conclude that, the hypothesis that a central component to fatigue which apparently exists during prolonged exercise, is not primarily mediated by serotonergic neurons.

The discrepant findings within the literature in relation to the original central fatigue hypothesis suggests that central serotonin function cannot be acutely manipulated by dietary or pharmacological intervention, despite some evidence of altered brain neurotransmitter function in some studies. Many studies then focussed their attention on central dopamine as a candidate for dietary manipulation. These studies adopted the revised central fatigue hypothesis suggested by Davis and Bailey (1997).

Table 1. Summary of studies examining effects of nutritional and pharmacological manipulation of neurotransmitters <i>in vivo</i>				
(See Table 2 for tyrosine studies)				
Animal Studies				
Authors	Animal	Treatment	Protocol	Main findings
Choi <i>et al.</i> , (2013)	Male Sprague-Dawley rats	BCAA / BCAA + Tyrosine	Sedentary and exercising rats provided treatment. Sacrificed 60 minutes after treatment ingestion.	BCAA reduced brain tryptophan and tyrosine concentrations. Lowered serotonin and catecholamine synthesis. Reductions in tyrosine concentration and catecholamine synthesis following BCAA intake can be reduced by co-administering tyrosine.
Hasegawa <i>et al.</i> , (2008)	43 male Wistar rats	17 mg·kg <sup>-1</sup> of a dual dopamine/noradrenaline reuptake inhibitor (bupropion)	Running to exhaustion at 26 m·min <sup>-1</sup> at 30°C and 18°C.	Running time to exhaustion in the heat was increased with bupropion. Extracellular concentrations of dopamine and noradrenaline significantly higher with bupropion.



Piacentini <i>et al.</i> , (2003)	Male Wistar rats	17 mg·kg <sup>-1</sup> dual dopamine/noradrenaline reuptake inhibitor (bupropion)	Samples for serotonin, dopamine, and norepinephrine were collected every 20 min before and after the injection of 17 mg·kg <sup>-1</sup> of bupropion for 180 mins.	Dopamine, noradrenaline and serotonin all increased after bupropion administration. Accompanied by decreased prolactin concentration.
Verger <i>et al.</i> , (1994)	34 male Wistar rats	BCAA, Glucose, Water	Running time to exhaustion.	Running time to exhaustion was highest following glucose administration. Rats fatigued quickest with BCAA ingestion.

Human Studies				
Authors	Population	Treatment	Protocol	Main findings
Bridge <i>et al.</i> , (2003)	12 active males	0.5 mg·kg body mass <sup>-1</sup> Buspirone alone, or in conjunction with 0.5 mg·kg body mass <sup>-1</sup> pindolol	Cycling at 73% $\dot{V}O_2$ max until exhaustion at 35°C.	Correlation between time to exhaustion and the non-serotonergic component of prolactin response to buspirone.
Cordery <i>et al.</i> , (2016)	10 physically active males	100 mg L-DOPA/25 mg carbidopa	1 hour cycling at 60% $\dot{V}O_2$ max followed by a 30 minute exercise test + finger tapping test and start and end of exercise.	No performance effect. Increasing catecholamine availability inhibits prolactin response.
Greer <i>et al.</i> , (2011)	9 untrained males	200 kcal of either BCAA, Carbohydrate or Placebo	3 x 90 minute bouts of exercise (per treatment) at 55% $\dot{V}O_2$ max followed by a 15 minute Time Trial.	CHO ingestion resulted in the greatest distance covered during the TT. BCAA had no effect. BCAA did positively affect RPE.

Pannier <i>et al.</i> , (1995)	8 healthy men	1 mg serotonin receptor antagonist pizotifen	Running at 70% $\dot{V}O_2$ max until exhaustion.	No effect of pizotifen on exercise capacity.
Piacentini <i>et al.</i> , (2002a)	7 well trained male cyclists	2 x 37.5 mg of venlafaxine	Cycling time trial	No effect of serotonin/noradrenaline reuptake inhibitor on exercise performance.
Piacentini <i>et al.</i> , (2002b)	7 well trained male cyclists	2 x 4 mg selective noradrenergic reuptake inhibitor reboxetine	Endurance time trial	Performance unaffected by reboxetine.
Roelands <i>et al.</i> , (2008)	8 healthy well trained males	20 mg dopamine reuptake inhibitor ritalin	Cycling for 60 minutes at 55 $W_{max}$ followed by time trial at 18°C and 30°C.	Ritalin improved exercise performance at 30°C but not 18°C.

Roelands <i>et al.</i> , (2008)	9 healthy well trained males	2 x 8 mg Reboxetine	Cycling for 60 minutes at 55 Wmax followed by time trial at 18°C and 30°C.	Norepinephrine reuptake inhibition reduced exercise performance in temperate and warm conditions.
Roelands <i>et al.</i> , (2009)	8 healthy well trained males	150 mg bupropion for 3 days following by 300 mg for 7 days.	Cycling for 60 minutes at 55 Wmax followed by time trial at 18°C and 30°C.	Bupropion increased circulating growth hormone levels. No effect of chronic bupropion administration on exercise performance.
Segura and Ventura (1988)	12 healthy sportsmen	4 x 150 mg tryptophan	Running to exhaustion at 80% $\dot{V}O_2$ max.	Exercise time was 49.4% longer following tryptophan ingestion. Perceived exertion was lower with tryptophan but not significant.

Strensrud <i>et al.</i> , (1992)	49 well trained male runners	Total of 1.2 g tryptophan administered over 24 hours prior to exercise bout.	Running to exhaustion at a speed corresponding to 100% $\dot{V}O_2$ max.	Tryptophan had no significant effect on time to exhaustion compared to placebo.
Strüder <i>et al.</i> , (1998)	10 males	21 g BCAA, 20 g tyrosine, 20 mg paroxetine.	Cycling time to exhaustion.	BCAAs and Tyrosine had no effect on time to exhaustion. Time to exhaustion was lower with paroxetine.
Watson <i>et al.</i> , (2006)	9 healthy endurance trained males	2 x 300 mg dual dopamine/noradrenaline reuptake inhibitor bupropion.	Cycling for 60 minutes at 55 Wmax followed by time trial at 18°C and 30°C.	Bupropion improves exercise performance at 30°C but not 18°C.

## **Tyrosine Supplementation, Behaviour and Cognitive Performance**

**(See summary table 2)**

### **Animal Studies**

There are a number of studies which have examined the effects of tyrosine administration on cognitive function in both animals and humans. Many of these studies provide evidence that tyrosine increases catecholamine metabolism under conditions of increased stress with maintenance, or the prevention of stress-induced declines, demonstrated in various cognitive performance measures.

Brady, Brown and Thurmond (1980) provided tyrosine to rats to assess its effect on aggressive behaviour, locomotor activity and brain neurochemistry following cold swim stress. Tyrosine administration increased aggressive behaviour in young, non-stressed mice while increasing levels of brain tyrosine and dopamine in both young and old mice. Tyrosine also prevented the decrease in locomotion in older stressed and non-stressed mice. Supplementation of tyrosine was found to be effective in reducing the effects of stress which induced an increased catecholamine metabolism (Brady, Brown and Thurmond, 1980).

Stress induced by tail shock was used by Lehnert, Reinstein, Strowbridge and Wurtman (1984) to assess tyrosine's effect on norepinephrine turnover, a key modulator in the stress response of mammals, and behavioural deficits in rats. Rats were provided with either a control diet or diets enriched with tyrosine or tyrosine + valine before exposure to tail shock or a controlled environment. Exposure to stress caused increased norepinephrine turnover resulting in norepinephrine depletion in some neurons. Behavioural deficits in rats were examined using measures of locomotion and exploration in an open field environment (Lehnert *et al.*, 1984).

Stressed rats displayed less exploration activity and spontaneous motor activity during the locomotion compared to control rats. Rats who received tyrosine did not display behavioural deficits nor a stress induced depletion in norepinephrine. However, rats who received tyrosine + valine did not demonstrate the same preventative effects as the tyrosine only group. This makes sense considering that valine competes with tyrosine for transport across the blood brain barrier (Lehnert *et al.*, 1984). It was also observed that when tyrosine was administered to rats not subjected to stress, there was no increase in norepinephrine turnover or behavioural responses. This evidence demonstrates that tyrosine prevents norepinephrine depletion and prevents behavioural deficits but only when catecholaminergic neurones are considerably more active as a result of stress exposure. Reinstein, Lehnert, Scott and Wurtman (1984) confirmed these results using a similarly designed study, once again finding tyrosine administration enhanced catecholamine synthesis and prevented behavioural deficits during stress exposure through tail shock.

Shurtleff, Thomas, Ahlers and Schrot (1993) administered 50, 100 or 200 mg·kg<sup>-1</sup> of tyrosine to eight rats to assess its effect on cognitive performance in a matching sample task. It was hypothesised that tyrosine supplementation would assist in preventing a deficit in brain catecholamine levels when exposed to acute environmental stress. The protocol involved the administration of one of the tyrosine doses or a saline solution (placebo) to the eight rats before the requirement to perform a delayed matching to samples task, within a temperature of either 22°C or 2°C. It was found that the higher doses of tyrosine (100 g and 200 g) were associated with improved cognitive performance during cold exposure in comparison to the lower 50 g dose and the placebo, demonstrating a possible dose-response effect. Specifically, it was found that matching accuracy was significantly improved and errors were significantly decreased in the cold at the higher tyrosine doses of 100 and 200 mg·kg<sup>-1</sup>. It was however found that while the higher

doses of tyrosine improved matching accuracy and reduced error rate in the cold, the results remained significantly decreased in comparison to those in the 22°C trial. It was concluded that improvements in the cold exposure trial were due to the amelioration of the deficit of brain catecholamine levels as a result of tyrosine administration.

The effect of tyrosine administration on behaviour in cold stressed rats was examined by Yeghiayan, Luo, Shukitt-Hale and Lieberman (2001). Rats were given tyrosine, amphetamine or phenylpropanolamine (an adrenoceptor agonist) alone or combinations of amphetamine or phenylpropanolamine + tyrosine to assess how tyrosine potentiates the drugs during 30 minute cold exposure and a subsequent swimming test. The study demonstrated that all three treatments improved performance and tyrosine + drug combinations further enhanced performance compared to single drug administration. Tyrosine also elevated brain norepinephrine concentration in rats stressed by hypothermia compared to rats treated with a saline solution.

Lieberman, Georgelis, Maher and Yeghiayan (2005) administered 400 mg·kg body mass<sup>-1</sup> tyrosine intraperitoneally to rats to assess its effect on norepinephrine synthesis during heat exposure (41°C). Memory and coping behaviour were assessed using a standardised maze and swim test respectively. Hippocampal dopamine and norepinephrine were also assessed using microdialysis. The study found that heat impaired coping and memory in vehicle treated rats and increased the release of norepinephrine. In rats treated with tyrosine, coping was not impaired and tyrosine sustained norepinephrine release as heat stress increased, demonstrating tyrosine to have a protective effect, through sustained norepinephrine release, during exposure to heat stress.

These studies demonstrate that tyrosine supplementation in rodents has a beneficial effect on *in vivo* behaviour, cognitive function and brain catecholamine synthesis across a range of different



environmental stressors.

## **Human Studies**

Prior to a number of studies examining the effects of tyrosine administration on both exercise capacity and performance as well as cognitive function, Glaeser, Melamed, Growdon and Wurtman (1979) demonstrated the effect of a single dose of tyrosine on plasma tyrosine concentration without an exercise protocol. Administration of either  $100 \text{ mg} \cdot \text{kg body mass}^{-1}$  or  $150 \text{ mg} \cdot \text{kg body mass}^{-1}$  produced increases in the tyrosine concentration from 69 to 154  $\text{nmols} \cdot \text{ml}^{-1}$  and to 203  $\text{nmols} \cdot \text{ml}^{-1}$ , respectively, 2 hours after ingestion. The tyrosine ratio, described in the study as the ratio of plasma tyrosine concentration to the sum of the concentrations of six other amino acids that compete for the same blood barrier uptake carrier (Glaser et al., 1979) increased from 0.10 to 0.28 in the  $100 \text{ mg} \cdot \text{kg}^{-1}$  dose and to 0.35 in the  $150 \text{ mg} \cdot \text{kg}^{-1}$  dose. Manipulation of amino acid ratios has also been demonstrated in previously mentioned study by Fernstrom and Wurtman (1972) which increased the ratio of tryptophan to competing amino acids, including tyrosine.

Similarly to animal studies, when tyrosine has been provided to human subjects, there appear to be improvements in cognitive function while exposed to stressful environments. Banderet and Lieberman (1989) provided  $100 \text{ mg} \cdot \text{kg body mass}^{-1}$  tyrosine to twenty three males prior to a 4.5 hour exposure to cold and hypoxia in a randomised crossover design. Tyrosine administration significantly reduced adverse emotions resulting from the environmental conditions. Tyrosine also improved vigilance, pattern recognition and reaction time. The study also demonstrated that tyrosine showed the greatest effect in participants who were most negatively affected by the environmental conditions.

Neri, Wiegmann, Stanny, Shappell, McCardie and McKay (1995) examined the behavioural effects of tyrosine during an episode of continuous work with a night of sleep loss. Twenty subjects performed a battery of performance tasks and mood scales for approximately 13 hours (Neri *et al.*, 1995). All participants remained awake on the day testing began and by the end of testing had been awake for greater than 24 hours. 6 hours into the experiment and in a double-blind fashion, half of the subjects received a dose of 150 mg·kg body mass<sup>-1</sup> tyrosine while the other half received a placebo. Tyrosine administration was shown to ameliorate the performance decline on a psychomotor task and demonstrated a reduction in lapse probability on a high event rate vigilance task (Neri *et al.*, 1995) demonstrating tyrosine to be successful in preventing performance decrements during sustained work and sleep loss (Neri *et al.*, 1995).

Deijen, Wientjes, Vullingsh, Cloin and Langefeld (1999) studied the effects of tyrosine on cognitive task performance. The participants used were all cadets who were involved in a demanding military combat training course. Out of the twenty one participants who were taking part in the study, ten received five daily doses of a protein-rich drink each of which contained 2 g of tyrosine. The remaining eleven subjects received a similar drink containing carbohydrate with a similar number of calories, but no tyrosine. Cognitive assessments were made prior to the beginning of the course and then on the sixth day. The results demonstrated that the group supplied with the drink containing tyrosine performed better on a memory and tracking task compared to the carbohydrate group when assessed on the sixth day of the course. A limitation, however, lies with the carbohydrate drink, which was acknowledged by the study as not a 'real placebo'. The macronutrient content of the drink may have resulted in increases in the blood ratio of LNAA: tyrosine, which may have increased synthesis of serotonin and release (Deijen *et al.*, 1998). It has been previously mentioned that carbohydrate, via insulin release, can lower the concentration of blood amino acids and therefore increase the tryptophan: LNAA ratio (Deijen *et*

*al.*, 1998). Despite this, the study concluded that ingestion of tyrosine under operational circumstances may assist in reducing the effects of stress and fatigue in cognitively demanding situations.

A study by Mahoney, Castellani, Kramer, Young and Lieberman (2007) used 19 participants to determine if tyrosine supplementation could improve human cognition when exposed to cold stress. Participants completed three trials on different days which required the completion of two 90-minute water immersions during each trial. Before each immersion, participants consumed a food bar which contained either 150 mg·kg body mass<sup>-1</sup> of tyrosine (300 mg·kg body mass<sup>-1</sup> total) or a placebo. The three immersion states were 35°C/control, 10°C/placebo and 10°C/tyrosine. During each trial, cognitive performance, mood and salivary cortisol were recorded. The study discovered that the cold water immersion resulted in a drop in cognitive performance although supplementation of tyrosine assisted in alleviating working memory decrements.

Lieberman *et al.*, (2014) examined whether tyrosine prevents the decline in cognitive ability in response to severe levels of stress induced by mock interrogations during a period of simulated captivity. The study recruited 78 male and female military personnel to participate who were administered either 300 mg·kg body mass<sup>-1</sup> of tyrosine or placebo. Tyrosine was administered via two food bars each containing 150 mg·kg body mass<sup>-1</sup> sixty minutes before participants were required to withstand a mock interrogation. Heart rate, saliva cortisol and mood (via profile of mood states questionnaire) were all recorded. The study found that tyrosine had no effect on the majority of responses to stress although it did demonstrate that supplementation of tyrosine increased ratings of anger. The study suggests that the feeling of anger may be an appropriate

response to stressful situations. For example, interrogations have been associated with a loss of control and optimism in healthy adults when they are exposed to stressful situations (Lerner *et al.*, 2007). This led to the conclusion that increased feelings of anger, brought about by tyrosine supplementation, may represent a coping mechanism to deal with stressful situations more effectively.

Coull *et al.*, (2015) examined whether a 150 mg·kg body mass<sup>-1</sup> dose of tyrosine would affect the cognitive and physical performance of eight male soccer players exercising in the heat. The implication was that exercise in the heat would be sufficiently stressful to increase the demands for brain tyrosine. The protocol involved each participant completing an individualised 90-minute simulated soccer performance test. This test was completed on two separate occasions in a temperature of 25°C and 60% relative humidity, once with the tyrosine dose and the other with a placebo. Mental effort and vigilance were also assessed. Participants were required to complete a dual task test with the requirement of tracking a moving target with the mouse cursor whilst, simultaneously, responding to varying stimuli with the spacebar. It was found that vigilance improved significantly with hit responses increased by  $9 \pm 28\%$  and miss responses decreased by  $31 \pm 29\%$  with tyrosine administration compared to placebo. The results suggested that tyrosine may have a positive effect on cognitive performance during exercise. A minor criticism of this study is that the protocol took place at an environmental temperature of 25°C; however, their data report that subjects were hyperthermic.

These studies provides evidence that tyrosine, in general, is an effective nutritional supplement in improving cognitive function when performing tasks in a wide variety of stressful environments, and using a wide range of doses.

## **Tyrosine Supplementation Alone and in Conjunction with Exercise**

**(See summary table 2)**

Studies in which humans were administered tyrosine to assess its effect on exercise performance and capacity have produced mixed results. Strüder, Hollman, Platen, Donike, Gotzmann and Weber (1998) conducted a study to assess the effects of 20 mg of paroxetine (serotonin reuptake inhibitor), 21 g of BCAA, 20 g of tyrosine or a placebo on constant load submaximal exercise to exhaustion in cyclists. Administration was carried out in a randomised, double-blind fashion, across four separate exercise trials. The tyrosine supplement was provided to participants at two separate occasions, the first 10 g was administered 15 minutes prior to the beginning of exercise while a further 10 g was provided after 60 minutes of exercise. Exercise capacity was significantly reduced when participants were administered paroxetine; there were no significant differences in time to exhaustion between the tyrosine trial, the branched chain amino acid trial or the placebo. It is possible that the exercise protocol was not physiologically or psychologically demanding enough to elicit a response from the tyrosine dose. This may have been caused by feedback inhibition of tyrosine hydroxylase in response to increased tyrosine availability in the brain. Had the exercise protocol been of a greater intensity or been carried out in a more stressful environment, the effects of tyrosine may have been more pronounced due to increased neuronal firing rates and a greater demand for tyrosine availability (Fernstrom, 1983; Brady *et al.*, 1980; Melamed *et al.*, 1980).

A study by Chinevere, Sawyer, Creer, Conlee and Parcell (2002) examined the effect of supplementing tyrosine alone, or in combination with carbohydrate, on endurance exercise performance in temperate environmental conditions. Nine competitive cyclists cycled for 90 minutes at 70% of their peak oxygen uptake which was immediately followed by a simulated

time trial. This protocol was conducted under four different feeding conditions which were randomly assigned to the participants, and included 5 mL·kg body mass<sup>-1</sup> of water (placebo), a 5 mL·kg body mass<sup>-1</sup> solution of polydextrose (carbohydrate), a 5 mL·kg body mass<sup>-1</sup> solution of tyrosine or a 5 mL·kg body mass<sup>-1</sup> solution with polydextrose and tyrosine (carbohydrate + tyrosine). No significant differences were found in time trial performance between the conditions, nor in subjective ratings, or physiological measures. Despite being unable to find any statistical differences in time trial performance; the results demonstrated that tyrosine did appear to influence the results of the study somewhat. Out of the nine participants taking part, six completed the time trial faster when supplemented with tyrosine compared to placebo. Furthermore, when participant were provided with a combination of tyrosine and carbohydrate, six out of nine participants completed the time trial faster compared to the other trials while eight out of the nine participants completed the time trial faster when provided with a combination tyrosine and carbohydrate compared to tyrosine or placebo alone. The study also found that ratings of perceived exertion were lower when participants were provided with the sole tyrosine solution. It is possible that, if the time trial had been longer or completed in the heat thus making it more demanding, the results may have shown a significant effect of tyrosine on time trial performance.

Sutton, Coll and Deuster (2005) examined how tyrosine ingestion affects endurance, muscle strength and anaerobic performance. Twenty men, all of whom were combat trained soldiers, completed two load carriage sessions to exhaustion on a treadmill following supplementation of either 150 mg·kg body mass<sup>-1</sup> tyrosine in 70 g of apple sauce or placebo. Following completion of the load carriage test, a physical performance battery was administered which included stair stepping (with weight), maximal and sub maximal handgrips and pull ups to exhaustion. On completion of the trials it was found that tyrosine supplementation had not significantly

improved treadmill performance time nor muscle strength and anaerobic performance. The study did find there to be a large increase in plasma tyrosine following tyrosine supplementation although the use of apple sauce in the process of supplementing the tyrosine may have affected the results due to the effect of carbohydrate (present in the apple sauce) on plasma amino acid ratios (Fernstrom and Wurtman, 1971).

Tumilty, Davison, Beckmann and Thatcher (2011) used eight males in their study to examine the effect of tyrosine supplementation on exercise capacity in the heat. Two exercise trials were performed by the participants in a randomised fashion, 1 hour after consuming a 500 mL drink containing either 150 mg·kg body mass<sup>-1</sup> tyrosine or a 500 mL placebo drink containing no tyrosine. Participants cycled to exhaustion at an exercise intensity of  $68 \pm 5 \text{ } \dot{V}O_{2\text{peak}}$  in a temperature of 30°C and 60% relative humidity. Tyrosine supplementation resulted in a 2.9 fold increase in the plasma ratio of tyrosine: competing amino acids which was not observed in the placebo trial. It was found that participants exercised for  $15 \pm 11\%$  longer with tyrosine compared with placebo despite values for core temperature, skin temperature, heart rate, ratings of perceived exertion and thermal sensation all being similar. The study was the first to demonstrate that acute tyrosine administration was associated with an increased capacity to endure exercise in the heat.

However, Watson, Enever, Page, Stockwell and Maughan (2012) also examined whether tyrosine supplementation affects exercise capacity in a warm environment, using a similar exercise protocol to the Tumilty *et al.*, (2011) study. Sixty minutes prior to exercise, participants consumed a 250 mL aliquot of tyrosine or placebo and then an identical 250 mL aliquot, 30 minutes prior to exercise, before cycling to exhaustion at 70%  $\dot{V}O_2$  max. Participants also

consumed either tyrosine or placebo after every 10 minutes of exercise had elapsed. The total amount of tyrosine consumed in the tyrosine trial was  $150 \text{ mg} \cdot \text{kg body mass}^{-1}$ . The study measured participant's cognitive function, using the Stroop word and colour test, Sternberg's memory scanning task and a rapid visual information processing task (Watson *et al.*, 2012). Cognitive function was measured before drink ingestion, immediately before exercise commenced and at exhaustion. Tyrosine supplementation had no effect on exercise capacity. The study did find that exercise caused an increase in error rate during the Stroop test but this was not influenced by the tyrosine supplement.

Other work by Tumilty, Davison, Beckmann and Thatcher (2013) explored the possibility of whether reduced plasma tyrosine and phenylalanine is associated with a reduced exercise capacity in the heat. The study again used eight male participants who completed two trials, consuming either 500 ml of sugar-free flavoured water containing either a balanced amino acid mixture containing 9 amino acids (isoleucine 15 g, leucine 22.5 g, valine 17.5 g, lysine 17.5 g, methionine 5 g, threonine 10 g, tryptophan 2.5 g, 12.5 g tyrosine and 12.5 g of phenylalanine) or the same mixture minus tyrosine and phenylalanine, designed to lower the blood ratio of plasma tyrosine and phenylalanine: competing amino acids. Subjects were required to cycle until exhaustion at  $63 \pm 5 \% \dot{V}O_2$  peak within a temperature of  $30^\circ\text{C}$  and 60% relative humidity. The study's results demonstrated that exercise time was shorter in the tyrosine-free trial compared to the balanced mixture trial. This study provided further evidence that the availability of tyrosine within the bloodstream can influence the ability to carry out prolonged, submaximal intensity exercise in the heat.

More recent work, published by Tumilty *et al.*, (2014) examined whether self-paced exercise performance in the heat is enhanced with tyrosine. Due to the nature of a self-paced time trial,



which is highly influenced by arousal and motivation, it was hypothesised that this would provide the necessary environment to highlight any apparent effects of tyrosine supplementation compared to a constant load capacity trial (Tumilty *et al.*, 2014). This study recruited 7 endurance trained males to perform two experimental trials in the heat (30°C and 60% relative humidity). Over the two trials, subjects consumed either 150 mg·kg body mass<sup>-1</sup> of tyrosine or a placebo in a randomised, crossover design. Following 60 minutes of rest after supplement or placebo administration, participants cycled for 60 minutes at  $57 \pm 4\%$  peak oxygen uptake before completing a time trial which required completion of an individualised work target quantity as quickly as possible. The study found that tyrosine supplementation increased the ratio of plasma tyrosine to other amino acids competing for uptake at the blood brain barrier 2.5-fold but found no change in the placebo trial. Power output throughout the time trial and performance were not improved following tyrosine administration. Thermal sensation, ratings of perceived exertion, core temperature, skin temperature and heart rate were similar between the two experimental conditions which indicated that ingestion of tyrosine did not influence the physiological response to self-paced exercise in the heat. The failure to find any significant effect of tyrosine on exercise performance led the authors to suggest that one possible reason why tyrosine was not effective may have been because tyrosine, as a general catecholamine precursor, could have augmented central noradrenaline activity (Tumilty *et al.*, 2014). Increased noradrenaline activity is associated with reduced exercise performance and is evident in the previously discussed study by Roelands *et al.*, (2008). Tumilty *et al.*, (2014) also suggests that the adoption of a range of tyrosine doses may identify whether or not a larger tyrosine dose has the ability to improve exercise performance in the heat.

One study which has used two different doses was published by Coull, Christmas, Watson, Horsfall and Taylor (2015). This study examined the effect of 150 and 300 mg·kg body mass<sup>-1</sup>

doses of tyrosine on blood concentrations. However, they only used the dose which increased blood tyrosine to the largest extent to examine its effect on cognitive and physical performance in the heat in a follow-up study. Therefore, it is still unclear what effect different doses of tyrosine may have on exercise in the heat. The exercise protocol, which included a 60 minute walk at 6.5 km·h<sup>-1</sup> followed by a 2.4 km time trial with a 25 kg backpack, was completed at 40°C and 30% relative humidity with cognitive function test before, during and after exercise. The study found tyrosine to have no significant effect on physical or cognitive performance despite a significant rise in serum tyrosine: competing amino acids.

Table 2. Summary of studies examining the effects of tyrosine supplementation on exercise, physiological responses and cognitive performance <i>in vivo</i>				
Animal Studies				
Study	Animal	Treatment	Protocol	Main findings
Acworth <i>et al.</i> , (1988)	Male Sprague-Dawley rats	50-200 mg·kg <sup>-1</sup> tyrosine.	Direct brain microdialysis	Dose related increase in extracellular dopamine.
Badaway and Williams (1982)	Male wistar rats	5 mg·kg <sup>-1</sup> tyrosine to 500 mg·kg <sup>-1</sup> tyrosine.	<i>In vivo</i> examination	Catecholamine synthesis enhanced in small tyrosine doses but ineffective at > 50 mg·kg.
Brady <i>et al.</i> , (1980)	Male CF-1 mice	Tyrosine or Casein diet.	Cold water swim at 2°C - 6°C	Increased aggressive behaviour in young mice. Tyrosine prevented decreases in locomotion in stressed and non-stressed older mice.  Tyrosine administration increased brain tyrosine and dopamine.

Choi <i>et al.</i> , (2013)	Male Sprague-Dawley rats	BCAA / BCAA + Tyrosine.	Sedentary and Exercising rats provided treatment. Sacrificed 60 minutes after treatment ingestion.	BCAA reduced brain tryptophan and tyrosine concentrations. Lowered serotonin and catecholamine synthesis. Reductions in tyrosine concentration and catecholamine synthesis following BCAA intake can be reduced with co administering tyrosine.
During <i>et al.</i> , (1988)	Sprague-Dawley rats	50–100 mg·kg body mass <sup>-1</sup> Tyrosine.	Partial lesioning of nigrostriatal neurons.	Increased brain dopamine concentration with tyrosine. Lesioning of nigrostriatal neurons increased effect of tyrosine on dopamine release in surviving neurons.
Lehnert <i>et al.</i> , (1984)	Sprague-Dawley rats	Tyrosine or tyrosine + valine or control.	Tail shock + locomotion and exploration task.	Tyrosine maintained brain norepinephrine turnover and prevented behavioural deficits.

Lieberman <i>et al.</i> , (2005)	98 Fischer rats	400 mg·kg body mass <sup>-1</sup> tyrosine.	Heat exposure at 41°C with coping and memory tasks.	Increased coping behaviour and maintained brain noradrenaline release with tyrosine.
Neri <i>et al.</i> , (1995)	20 males	150 mg·kg body mass <sup>-1</sup> tyrosine or placebo.	Sleep loss + cognitive performance tasks.	Tyrosine prevented performance decrements.
Shurtleff <i>et al.</i> , (1993)	8 Long Evans rats	50, 100, 200 mg·kg <sup>-1</sup> tyrosine.	Delayed matching to sample task at 2°C and 22°C.	Tyrosine partially improved DMTS performance during cold stress.
Yeghiyayan <i>et al.</i> , (2001)	Male Fischer rats	Tyrosine (alone), amphetamine or phenylpropanolamine alone or with tyrosine (200 or 400 mg·kg body mass <sup>-1</sup> ). <sup>1</sup> ).	Cold immersion and forced swim test.	Tyrosine increased brain norepinephrine concentration. All treatments improved performance. Performance further enhanced with addition of tyrosine.

Human Studies				
Study	Population	Treatment	Protocol	Main findings
Banderet and Lieberman (1984)	23 males	100 mg·kg body mass <sup>-1</sup> tyrosine.	4.5 cold and hypoxia exposure.	Tyrosine improved adverse mood and cognitive function in subjects most affected by environment.
Chinevere <i>et al.</i> , (2002)	9 competitive cyclists	25 mg·kg body weight <sup>-1</sup> tyrosine or 25 mg·kg body mass <sup>-1</sup> tyrosine with 70 g·l <sup>-1</sup> polydextrose.	Cycling at 70% $\dot{V}O_{2peak}$ for 90 minutes followed by a time trial.	Tyrosine had no effect on time trial performance following 90 minutes of submaximal exercise.
Coull <i>et al.</i> , (2014)	8 male soccer players	150 mg·kg body mass <sup>-1</sup> tyrosine.	Individualised 90 minute soccer simulation intermittent soccer performance test at 25°C.	Tyrosine associated with improved vigilance and mental effort.  Tyrosine may improve cognitive function during heat stress.

Coull <i>et al.</i> , (2015)	21 males	2 doses of 150 mg·kg body mass <sup>-1</sup> tyrosine or 2 doses 75 mg·kg body mass <sup>-1</sup> tyrosine.	Military based load carriage protocol including 60 minute walk followed by 2.4 km time trial with 25 kg backpack at 40°C.	Single dose of 150 mg·kg body mass <sup>-1</sup> tyrosine as efficient at elevating serum tyrosine concentration as double dose. No effect of tyrosine in alleviating impaired cognitive function.
Deijen <i>et al.</i> , (1998)	21 military cadets	5 x doses of 2 g tyrosine.	Cognitive tasks including a memory comparison task, tracking task, continuous memory task, double task.	Tyrosine may improve cognitive task performance by reducing stress under operational circumstances.

Lieberman <i>et al.</i> , (2014)	78 male and female military personnel	2 x 150 mg·kg body mass <sup>-1</sup> tyrosine.	2 mock interrogations during several days of simulated captivity at SERE school. Mood, cortisol and heart rate measured.	Tyrosine increased ratings of anger during simulated captivity.
Mahoney <i>et al.</i> , (2007)	19 male and female volunteers	2 x 150 mg·kg <sup>-1</sup> tyrosine.	2 x 90 minute cold water immersions to induce a decrease in core temperature. Cognitive performance, mood and salivary cortisol analysed.	Tyrosine resulted in improved match to sample memory measure and improved response time demonstrating more accurate information processing.



Steenbergen <i>et al.</i> , (2015)	22 healthy adults	2 g tyrosine dissolved in 400 ml orange juice administered in 2 separate doses.	Participants rated mood on a 9 x 9 pleasure x arousal grid before administration of tyrosine. Mood was rated again following tyrosine administration before completing the task switching paradigm. Mood was then rated for a third time.	Tyrosine promotes cognitive flexibility. Tyrosine facilitates cognitive flexibility by replenishing cognitive resources.
Strüder <i>et al.</i> , (1998)	10 males	21 g BCAA, 20 g tyrosine, 20 mg paroxetine.	Cycling time to exhaustion.	BCAAs and tyrosine had no effect on time to exhaustion. Time to exhaustion was lower with serotonin reuptake inhibitor (paroxetine).

Sutton <i>et al.</i> , (2005)	20 males	150 mg·kg body mass <sup>-1</sup> tyrosine administered in apple sauce.	2 load carriage sessions followed by a physical performance battery.  Including maximal and submaximal handgrip, pull-ups, and stair stepping with additional weight.	Total time on treadmill was unaffected by tyrosine ingestion.  Physical performance battery was also unaffected by tyrosine.
Tumilty <i>et al.</i> , (2011)	8 healthy males	150 mg·kg body mass <sup>-1</sup> tyrosine.	Cycling to exhaustion at  68 ± 5 % $\dot{V}O_{2peak}$ at 30°C.	Time to exhaustion increased with tyrosine despite similar values for core temperature, heart rate, thermal sensation and RPE.

Tumilty <i>et al.</i> , (2014)	7 endurance trained males	150 mg·kg body mass <sup>-1</sup> tyrosine.	60 minute cycling at 57 ± 4 $\dot{V}O_{2peak}$ followed by a cycling time trial.	Tyrosine had no effect on cycling performance in comparison to placebo despite marked rises in plasma tyrosine.
Watson <i>et al.</i> , (2012)	8 males	150 mg·kg body mass <sup>-1</sup> tyrosine.	Cycling to exhaustion at 70% $\dot{V}O_{2peak}$ with cognitive function tests at before drink ingestion, at the end of 1 hour rest period and at exhaustion.	Tyrosine did not influence time to exhaustion despite increase in serum tyrosine and had no effect on cognitive function.

There are no published studies which have examined the effect of varying doses of tyrosine in humans, before prolonged exercise in the heat. Coull *et al.*, (2015) initially used a high and low dose in the primary stages of their study in order to identify an optimal dose for increasing serum tyrosine but did not systematically examine the effect of both doses on exercise and cognitive function. Many studies, such as Sutton *et al.*, (2005), Tumilty *et al.*, (2011), Watson *et al.*, (2012), Tumilty *et al.*, (2014) and Coull *et al.*, (2014) have all favoured a dose of 150 mg·kg body mass<sup>-1</sup> in their studies. These studies report large increases in the blood ratio of tyrosine: LNAA but the conflicting results with the adoption of this dose could suggest that this is insufficient to maintain brain tyrosine concentrations in all individuals, under the physiologically demanding conditions of exercise and heat. Previous studies which have adopted a higher dose than 150 mg·kg body mass<sup>-1</sup>, such as Mahoney *et al.*, (2007; 300 mg·kg body mass<sup>-1</sup>), reported improved cognitive function during exposure to cold stress. Therefore, it is prudent to investigate whether the acute dose of tyrosine administered may influence exercise performance in the heat. Administering higher doses of tyrosine may maximise the increase in the blood ratio of tyrosine: LNAA, favouring entry of tyrosine into the brain. Maximising brain tyrosine should, in theory, result in an increase in the synthesis of dopamine in the brain assuming that exercise combined with heat stress, is sufficiently demanding to stress brain catecholamine function. Previous studies have shown that tolerance to exercise in the heat can be predicted by dopaminergic activity within the hypothalamus (Bridge *et al.*, 2003), providing some support for this. Increases in neuronal firing will result in a decrease in precursor availability and so additional tyrosine should maintain catecholamine synthesis and release (Fernstrom, 1983). The additional tyrosine should, in theory, maintain brain catecholamine activity and subsequently maintain, or improve performance during exercise in the heat.

## **Aims and Hypothesis**

The present study aims to assess the effect of a low, medium or high dose of tyrosine on exercise performance in the heat. It is hypothesised that tyrosine will improve performance in the medium and high dose as these should increase the ratio of tyrosine: LNAA to a greater extent compared to the low dose. The low dose to be used in the present study has produced mixed results to date and therefore it is plausible to suggest that the medium and high dose of tyrosine will increase the ratio of tyrosine: LNAA to a greater extent. Therefore, this will provide greater precursor availability for catecholamine synthesis under conditions of increased neuronal firing. This may subsequently improve exercise performance compared to a low dose of tyrosine and placebo.

## **Method**

### **Participants**

All participants volunteered to take part and were given written information on the purpose and procedures of the study before giving verbal and written informed consent to proceed. Participants completed a health questionnaire prior to commencing the study in order to identify any reason that would preclude involvement. Nine healthy males, all of whom exercised regularly, volunteered to participate. None of the participants were specifically acclimated to exercise in the heat. One participant withdrew from the study having completed two laboratory visits and the remaining eight participants completed all testing. Data for eight participants were used for statistical analysis. The physical characteristics of these participants were: mean age,  $23 \pm 4$  (SD) years; height,  $181 \pm 7$  cm; body mass,  $76.1 \pm 5.9$  kg; peak oxygen uptake ( $\dot{V}O_2$  peak) obtained from ramp incremental test,  $4.1 \pm 0.5$  L·min<sup>-1</sup>; peak power achieved during ramp test,  $327 \pm 44$  W.

Ethical approval for the study was granted by Aberystwyth University Research Ethics Committee prior to any subject recruitment or testing procedures taking place. All the participants who took part were informed that participation was voluntary and they were free to withdraw from the study at any point without giving prior notice.

### **Experimental Protocol**

Participants attended the laboratory on six separate occasions in the morning, having refrained from the consumption of alcohol and any strenuous or unaccustomed physical activity for 24 hours prior to each visit. They were asked to arrive at the lab well rested, and to sleep for a minimum of 8 hours the night before each visit. Verbal adherence to this was given on each visit.

Furthermore, participants recorded their diet for 24 hours prior to the familiarisation and repeated their dietary intake before each subsequent experimental visit. Participants attended each trial after an overnight fast of at least 8 hours (not including the  $\dot{V}O_2$ peak test, see below) but were instructed to consume 500 mL of plain tap water, 2 hours before arriving at the lab for each visit. The first visit required participants to complete a ramp incremental exercise test on a cycle ergometer (Lode Excalibur Sport 2, Groningen, Netherlands) to measure  $\dot{V}O_2$ peak, using an online breath by breath system (Quark PFT, Cosmed, Rome, Italy). At least 48 hours later, participants completed a familiarization visit to become accustomed to the experimental procedures, including exercising in the heat, and to minimise any learning effects. The familiarisation trial was identical to the placebo trial, and included administration of the placebo drink (see below) but no blood sampling was performed. Finally, each participant completed the remaining four trials in a randomised, crossover fashion, with at least 7 days between each visit. Trial order was randomised using an open source software package (PEPI for Windows, Brixton Health). In three of the trials participants consumed tyrosine in one of the three experimental doses (Low, Medium or High) and in the fourth trial the placebo drink was administered. Administration of the experimental and placebo drinks was double-blinded and all drinks were prepared by a separate drink manager, not directly involved in data collection.

The tyrosine drinks (SHS International, UK) provided a total of 150 (Low), 300 (Medium) or 400 (High) mg·kg body mass<sup>-1</sup> tyrosine. All drinks were administered in two separate bottles which were consumed an hour apart. The 300 mg·kg dose was administered as two bottles each containing 300 mL sugar-free flavoured squash (Morrisons, Bradford, UK) and tap water and 150 mg·kg body mass<sup>-1</sup> tyrosine whereas the 400 mg·kg dose was administered as two bottles each containing 300 mL sugar-free lemon and lime flavoured squash and tap water and 200 mg·kg body mass of tyrosine. The 150 mg·kg dose was administered as two bottles, the first

containing 300 mL sugar-free flavoured squash and tap water and 150 mg·kg body mass of tyrosine and the second bottle containing the same fluid but no tyrosine. The placebo dose was administered in two bottles and contained the same fluid volume and constituents as the experimental drinks but no tyrosine.

### **Maximal Oxygen Uptake Test**

All participants completed a 30 W·min<sup>-1</sup> ramp test to exhaustion on a cycle ergometer to establish their  $\dot{V}O_2$  peak. Prior to each peak oxygen uptake test, the breath-by-breath analyser was calibrated using a standard 3 L volume cylinder (Hans Rudolf, Kansas, USA) and gases of known concentration, assuming accuracy of the concentration certificates provided by the supplier (BOC, Guildford, UK). Participant's height (Holtain Ltd, Crymych, UK) and nude body mass (Seca 899, Hamburg, Germany) were recorded before being fitted with a radiotelemetry heart rate monitor (Polar, FS2C, Kempele, Finland). Once seated on the cycle ergometer seat height and handle bar position was adjusted into the appropriate position for each participant and recorded for replication in subsequent visits. Participants were fitted with a sterilised silicon face mask (Hans Rudolf, Kansas, USA), which was held in place via a head cap (Hans Rudolf, Kansas, USA), and sat quietly on the ergometer for one minute to regulate their breathing. The first three minutes of exercise were unloaded (0 W) then the power output increased by 1 W every 2 seconds until participants reached volitional exhaustion. No information relating to performance was visible to the participant and strong verbal encouragement was provided throughout.

Following completion of the  $\dot{V}O_2$  peak test, the data was averaged over six seconds and was exported to Microsoft Excel (Microsoft Excel 2010, Computer Software, USA). Each



participant's gas exchange threshold (defined as an increase in the rate at which  $\dot{V} \text{CO}_2$  rises versus  $\dot{V} \text{O}_2$ , accompanied by an increase in  $\dot{V} \text{E}/\dot{V} \text{O}_2$  while  $\dot{V} \text{E}/\dot{V} \text{CO}_2$  continued to decrease or levelled off) was determined through the use of modified version of the v-slope method (Beaver *et al.*, 1985). Data for  $\dot{V} \text{O}_2$  and  $\dot{V} \text{CO}_2$  were plotted against time in order for the gas exchange threshold to be visually identified and were confirmed by a graph of ventilatory equivalents versus time. Identification of the gas exchange threshold for each subject was confirmed by an additional, separate researcher. The power output equivalent to 10%  $\Delta$  (Power output equivalent to 10% of the difference between the  $\dot{V} \text{O}_2$  at the gas exchange threshold and the  $\dot{V} \text{O}_{2\text{peak}}$ ) was calculated individually for each subject from the gas exchange data and adopted for the constant-load, submaximal portion of the exercise protocol (described below).

## **Experimental Trials**

All familiarisation and experimental trials commenced between 0700 and 0800 hours and at the same time of day for each participant, across trials. Subjects were required to fully empty their bladder into a 1 L Pyrex beaker (Fisher Scientific UK Ltd., Loughborough, UK) and provide their nude body mass. Urine volume was determined using a 500 mL measuring cylinder (Fisher Scientific UK Ltd., Loughborough, UK) before urine osmolality was measured, on arrival at the lab for each visit, via a digital refractometer (Pocket Osmocheck 4595, Vitech Scientific, West Sussex, UK), to ensure participants were not hypohydrated (urine osmolality > 700 milliosmoles per kg; ACSM, 2007). If it was found that participants were hypohydrated, testing was rescheduled by a minimum of 24 hours to the next available day convenient for the participant and investigator. Participants were instructed to drink fluids regularly to ensure rehydration prior to returning to the lab for any rescheduled visits. Approximately 1 ml of urine was immediately frozen for subsequent measurement via freezing point depression and this data is reported below

(Osmostat 030, Gonotec, Berlin). The analyser was calibrated using calibration fluids of known osmolality (0, 300, and 850 mosmol·kg<sup>-1</sup>). Participants were asked to fit a rectal thermistor (Grant Instruments, Cambridge, England, UK) 10cm beyond the anal sphincter, in a private room, as well as fit a heart rate telemetry band (Polar S610i, Polar Electro Oy, Tampere, Finland) on the chest. Skin thermistors (Grant Instruments, Cambridge, England) were attached to the participant's right calf, halfway between the ankle and the knee; to the right anterior thigh, halfway between the knee and hip; right upper section of chest, midway between the nipple line and the clavicle; and on the right arm, midway between the shoulder and the elbow, over the tricep. The surface skin probes were securely attached to the skin at the thermistor head using breathable adhesive medical tape (Hypafix, Bsn Medical, Hull, UK) and then connected to a data logger (Squirrel SQ2020, Grant Instruments, Cambridge, England). The recorded values for skin temperature were used to calculate mean weighted skin temperature using the equation devised by Ramanathan (1964):

$$0.3 \cdot (T_{chest} + T_{arm}) + 0.2 \cdot (T_{thigh} + T_{leg}) \quad [\text{equation 1}]$$

where  $T_{chest}$  is temperature at the chest site,  $T_{arm}$  is temperature at the arm site,  $T_{thigh}$  is temperature at the thigh site and  $T_{leg}$  is temperature at the calf site.

Participants sat quietly for a minimum of 15 minutes prior to a 6 mL blood sample (Rest) being obtained from the antecubital vein and collected into a heparinised vacutainer (BD Vacutainer Systems, Plymouth, UK), with minimal stasis, using standard venepuncture methods. Following drink ingestion, participants sat quietly in the laboratory for one hour. During this period, core, thigh, calf, chest and arm temperature, heart rate, air temperature and humidity were all recorded every 5 minutes (Squirrel SQ2020, Grant Instruments, Cambridge, England). Thermal sensation

was also recorded every 5 minutes, using a validated scale ranging from -10 'Cold impossible to bear' to +10 'Hot impossible to bear' (adapted from Parsons, 2003). After 60 minutes, a second blood sample was obtained (Pre) using exactly the same procedure described above, and then participants drank a second 300 mL of sugar-free squash and tap water containing tyrosine or the placebo. Drinks were provided to the participant in an opaque plastic bottle and shaken well before consumption to mask any differences in taste, texture and colour. Prior pilot work conducted with two volunteers, who did not participate in the study, confirmed that the drinks were indistinguishable in texture and taste.

Participants entered the climate chamber (Design Environmental, Gwent, Wales), which was maintained at a temperature of 30°C and 60% relative humidity [mean wind speed within chamber was 0.6 mph, measured using a hand-held anemometer (Kestrel 1000, Richard Paul Russell Ltd, Lymington, UK)], and positioned themselves on the cycle ergometer (Lode Excalibur Sport 2, Groningen, Netherlands). Immediately before exercise commenced, baseline measures were taken for core and skin temperature, heart rate and thermal sensation. Air temperature and humidity were also recorded to the nearest 0.1°C and 0.1% respectively, by the chamber control software and wind speed was monitored within the chamber using the hand-held anemometer. Measurement of wind speed was calculated as the mean of four measurements taken at head height in front, behind, to the left and to the right of each participant as they were seated on the cycle ergometer. Participants began cycling, with no prior warm up, at a constant intensity equivalent to 10%  $\Delta$  for 60 minutes ( $129 \pm 17$  W and  $47.8 \pm 6.02$  %  $\dot{V}O_2$  peak in this group). The purpose of the 60 minute exercise period was to induce hyperthermia prior to the start of the time trial. Participants were given no feedback on time elapsed. Core and skin temperature, heart rate, thermal sensation, ratings of perceived exertion (Borg, 1982), air temperature, humidity and wind speed were recorded after every 5 minutes of exercise and in the

last few seconds of the 60 minute exercise period. Drinks were provided to the participants (2 mL·kg body mass<sup>-1</sup> tap water with 20% sugar free lemon and lime squash) after 15, 30, 45 and 59 minutes of exercise had elapsed. Following completion of the 60 minute exercise period, participants dismounted the cycle ergometer and were quickly moved to a chair, which was positioned inside the heat chamber directly adjacent to the bike, where a third venous blood sample was obtained (Post 60). Acquisition of the Post 60 blood sample occurred within two minutes in all cases. Participants remounted the cycle ergometer and performed a simulated cycling time trial. The time trial in the present study has been used previously in our lab (Tumilty *et al.*, 2014) and is based on an original validated time trial protocol by Jeukendrup *et al.*, (1996). The time trial required participants to complete a quantity of work ( $326 \pm 37$  kJ in this group) as quickly as possible, equivalent to 30 minutes of cycle exercise at 60% of the power output which elicited  $\dot{V}O_2$  peak during the initial ramp test. Work target quantities were calculated prior to familiarisation and experimental time trials. During the time trial, the cycle ergometer was set in linear mode so that power output, and work accumulated, was related directly to pedalling cadence. The linear factor for each participant was calculated using the equation:

$$LF = \frac{0.6 \cdot PO \text{ at } \dot{V}O_{2\text{peak}}}{RPM^2} \quad [\text{Equation 2}]$$

where LF is Linear Factor, PO at  $\dot{V}O_{2\text{peak}}$  is the power output (W) which elicited  $\dot{V}O_{2\text{peak}}$  during the ramp test and RPM is the subject's preferred pedal cadence at this power output.

The preferred cadence at 60%  $\dot{V}O_2$  peak for each participant, which was used in subsequent linear factor calculations, was identified during the familiarisation trial. Between 48 and 50 minutes of the 60 minute cycling period, participants were instructed to maintain their preferred cadence as the power output was increased to each individual's 60% peak ramp power value.

Before commencing, participants were instructed to complete the time trial as quickly as possible but no further motivational encouragement was provided. Participants could view total work accumulated from the bike console, which was positioned on the frame of the bike and in view of the participant. No feedback was provided on power output, time elapsed or pedalling cadence. Information on cumulative work targets was visible on a laminated card positioned on the bike handlebars, and displayed the target work required to complete 25%, 50%, 75% and 100% of the individualised work target. Participants were permitted to drink *ad libitum* during the time trial (tap water with 20% sugar free lemon lime squash) and the volume of fluid consumed was recorded during each trial. Following the completion of the time trial, participants were quickly moved back to the adjacent chair within the chamber and a final blood sample was taken (PTT), within a maximum of two minutes. Participants were immediately removed from the climate chamber and sat in a cool environment for 15 minutes to recover. During this period, core and skin temperature, heart rate, thermal sensation, air temperature and humidity were recorded every 5 minutes. Once complete, the skin thermistors were removed and the rectal thermistor was removed by the participant in private, before emptying their bladder so post exercise urine volume and osmolality could be recorded. A further 1 ml of urine was frozen for osmolality measurement using freezing point depression. Finally, nude body mass was recorded a second time. Changes in participant's body mass which occurred during exercise were calculated as the difference between pre exercise and post exercise weight, accounting for fluid consumption and urine output. Participants were then free to shower before leaving the laboratory.

## Blood Treatment

A 1 mL aliquot of whole blood was transferred from the heparinised vacutainer into a 1.5 mL eppendorf. Blood glucose and blood lactate were analysed using an automated glucose/lactate analyser (2300 Stat Plus, Yellow Spring Instrument Co., Ohio, USA). The analyser was calibrated regularly with standard concentrations for glucose (0.00, and 50.00 mmol·L<sup>-1</sup>) and lactate (0.00 and 30.00 mmol·L<sup>-1</sup>). The coefficient of variation for 10 repeated measurements on a sample standard of known concentration for blood glucose (6.14 mmol·L<sup>-1</sup>) and blood lactate (5.35 mmol·L<sup>-1</sup>) were 1.7% and 1.5%, respectively (Tumilty, 2011). The remaining whole blood was centrifuged at 1500 g for 10 minutes at 4°C. The separated plasma was pipetted evenly into two separate eppendorfs and frozen at -80°C for subsequent analysis of amino acids using gas chromatography mass spectrometry (GC-MS). Haemoglobin concentration was measured using an automated haematology analyser (ABX Pentra 60C+, Horiba ABX Diagnostics, Northampton, UK). This method of analysis has a coefficient of variation of 0.3%, for 10 repeated measurements on the same sample standard (Tumilty, 2011). Haematocrit was measured using standard microcentrifugation. Whole blood from the 1 mL taken from the vacutainer was drawn into three separate glass capillary tubes and spun for 5 minutes at 14000 g using a micro-centrifuge (Haematospin 1400, Hawksley, Lancing, UK). Individual tubes were then placed into a Hawksley micro-haematocrit reader (Hawksley, Lancing, UK) and red blood cell mass was recorded. This technique had a coefficient of variation of 0.8% for ten repeated measurements on the same sample (Tumilty, 2011). Measurements of blood glucose, blood lactate and haemoglobin were measured in duplicate and haematocrit was measured in triplicate.

## Amino Acid Analysis

Amino acid analysis was completed by a separate investigator with the methods used identical to previous studies of similar design (Tumilty, 2011) to the present investigation. GC-MS was used to measure plasma amino acids concentrations in heparinised plasma. From ice, 200  $\mu\text{L}$  of defrosted plasma samples were transferred into  $\sim 500$   $\mu\text{L}$  micro tubes which contained glass beads (2 mL Eppendorf), diluted with 1520  $\mu\text{L}$  degassed (He) and chilled ( $-20^{\circ}\text{C}$ ) methanol/chloroform (4:1, v/v), vortexed for 10 seconds, shaken for 15 minutes at  $4^{\circ}\text{C}$  and then centrifuged for 5 minutes at  $4^{\circ}\text{C}$  and 18000 g (Hettich EBA 12R, Tuttlingen, Germany; Tumilty, 2011). For GC-MS analysis, 420  $\mu\text{L}$  of the supernatant was transferred into separate 2 mL micro tubes and dried in vacuo (speed vac, Univapo 150 H). The sample tubes containing supernatant were then stored at  $-80^{\circ}\text{C}$  (Tumilty, 2011). Two-step derivatization of dried samples was achieved by protecting the carbonyl moieties by methoximation using 60  $\mu\text{L}$  of a 20  $\text{mg}\cdot\text{mL}^{-1}$  solution of methoxyamine hydrochloride (Fluka) in pyridine (Fluka) at  $30^{\circ}\text{C}$  for 90 minutes (Tumilty, 2011). Acidic protons were then derivatized with 60  $\mu\text{L}$  N-methyl-N-trimethylsilyltrifluoride (MSTFA, M&N) at  $37^{\circ}\text{C}$  for 30 minutes. Sixty  $\mu\text{L}$  were transferred into 200  $\mu\text{L}$  glass vials (Chromacol) and 1  $\mu\text{L}$  were injected split-less into a Leco Pegasus III GC-tof-MS system (St. Joseph, USA) consisting of a Focus autosampler (Anatune), an Agilent 6890 N gas chromatograph equipped with a DB5-MS column (20 m x 0.25 mm ID x 0.25  $\mu\text{m}$  film; Tumilty, 2011). Injector temperature was  $250^{\circ}\text{C}$  with the transfer line set to  $260^{\circ}\text{C}$  and the ion source temperature held at  $230^{\circ}\text{C}$ . Helium flow was  $1.2\text{ mL}\cdot\text{min}^{-1}$ . Following 1 minute at  $80^{\circ}\text{C}$ , oven temperature was increased by  $30^{\circ}\text{C}\cdot\text{min}^{-1}$  to  $330^{\circ}\text{C}$ , maintained at  $330^{\circ}\text{C}$  for 3 minutes and then decreased to  $80^{\circ}\text{C}$  (Tumilty, 2011). Automated deconvolution and peak finding was completed using ChromaTof software (Leco, St. Joseph, USA). Through use of this technique, the coefficient of variation for the measurement of individual amino acids was: leucine, 8.5%;

isoleucine, 9.7%; valine, 5.8%; methionine, 11.4%; tryptophan, 5.2%; phenylalanine, 7.5% and tyrosine, 6.5% (Tumilty, 2011).

### **Statistical Analysis**

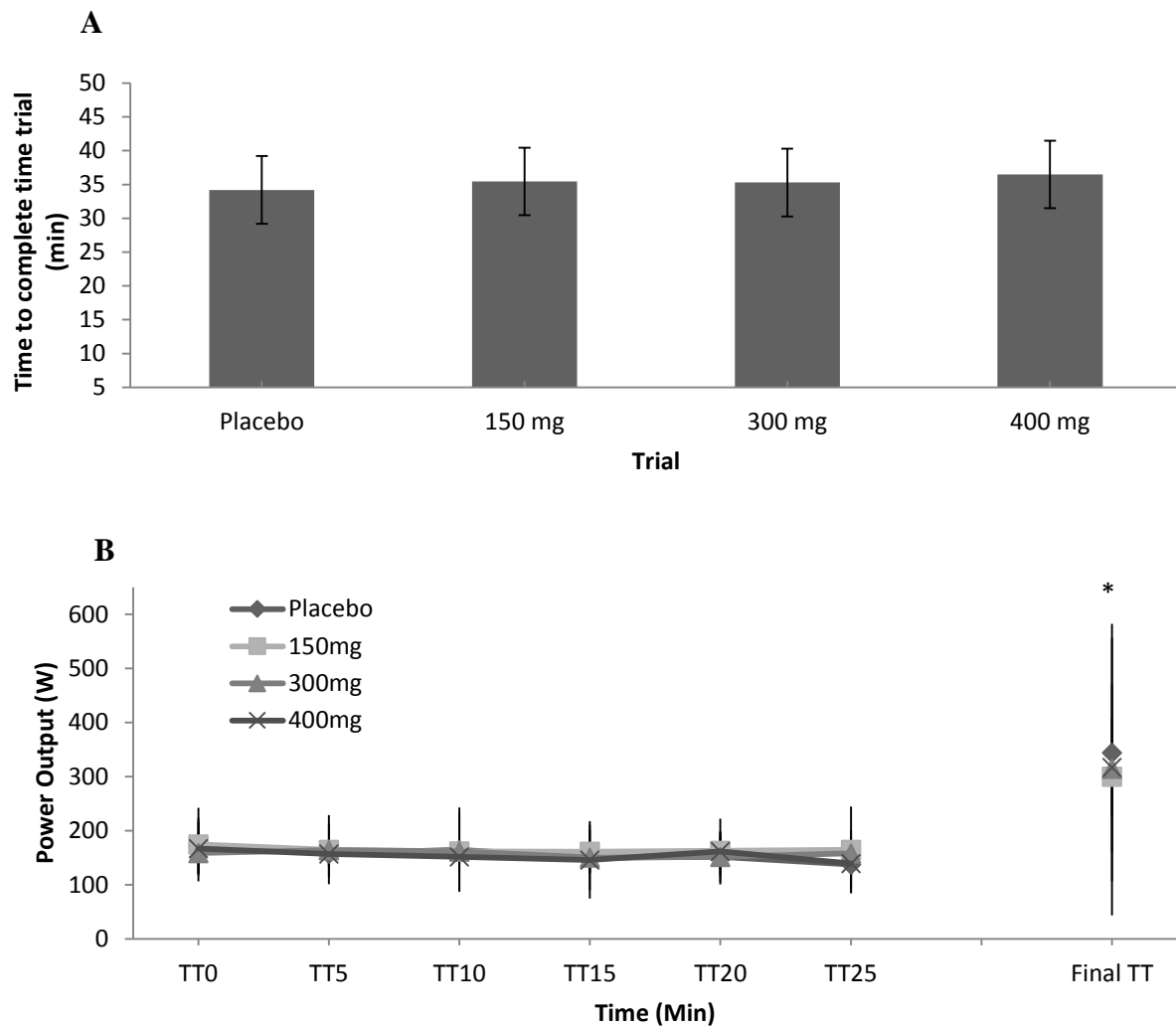
A computerized statistical package was used to analyse all data (SPSS version 17.0, SPSS inc., Chicago, IL). Normally distributed data are presented as mean  $\pm$  SD. Normally distributed data throughout the trials were compared using 2-way (time x trial) repeated measures ANOVA. Follow up *post hoc* tests were used, where appropriate, using Student's paired T-tests with the Bonferroni correction. Non-normally distributed data were analysed using Friedmans Test and are presented as median (range). Where necessary, *post hoc* tests were carried out using Wilcoxon signed rank tests with the Bonferroni correction. Statistical significance was accepted at  $P < 0.05$ .



## Results

### Time trial performance

After completing all trials, three participants successfully guessed the trial in which they received the placebo, stating differences in the drinks texture, although were unable to distinguish between the different doses of tyrosine. The remaining five participants were unable to distinguish between the tyrosine doses or placebo. This indicates successful drinks blinding. Tyrosine did not influence the time taken to complete the time trial ( $P = 0.588$ ;  $34.2 \pm 2.5$  min in PLA,  $35.4 \pm 5.9$  min in LOW,  $35.2 \pm 5.2$  min in MED and  $36.4 \pm 5.9$  min in HIGH; Figure 1A). There were no significant differences in median power output between conditions at any point during the first 25 minutes of the time trial ( $P = 0.281$ ). There was no effect of trial order ( $P = 0.844$ ).



**Figure 1. Mean ( $\pm$  SD) time to complete time trial (A) and median (range) power output (B) up until 25 mins of time trial had elapsed (last common time-point when all participants were still exercising) and at end of exercise, in the placebo trial (PLA), 150 mg·kg (LOW), 300 mg·kg (MED) and 400 mg·kg (HIGH) tyrosine trials. (n = 8). \* Significant difference in final TT values compared to power output values up to 25 mins in PLA, MED and HIGH.**

## Blood Analysis

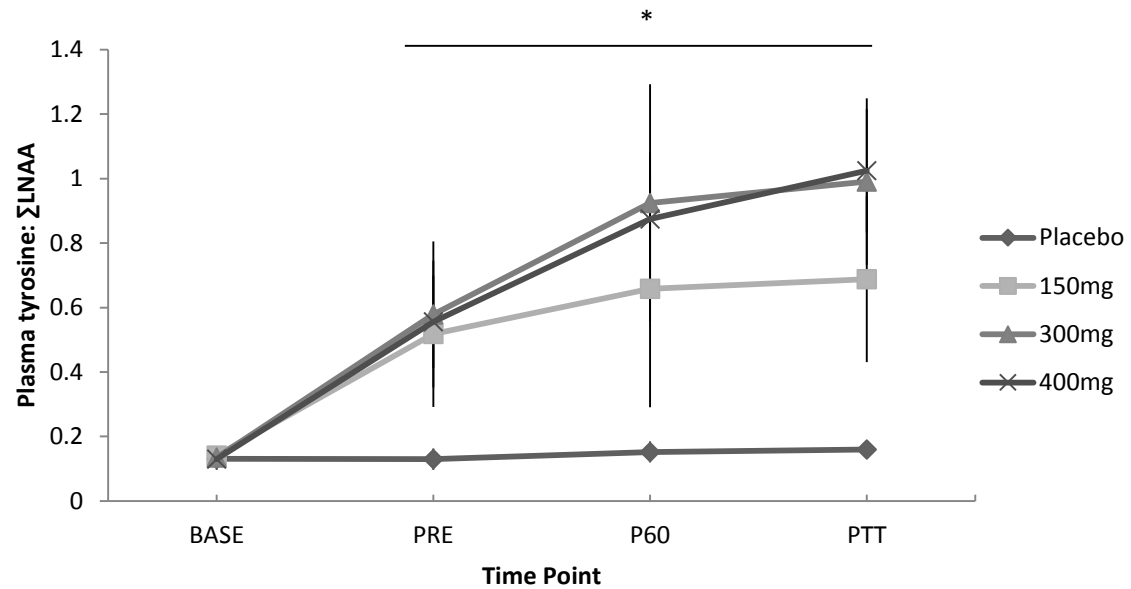
For clarity, caps on error bars have been removed in all the time-series graphs. All blood measures were adjusted for plasma volume changes. The plasma ratio of tyrosine:  $\sum$ LNAA (sum of the plasma concentration of tryptophan, valine, leucine, isoleucine, phenylalanine and methionine) was similar at rest between all trials ( $P = 0.657$ ) but increased significantly from rest in all tyrosine conditions ( $P < 0.01$ ; Figure 2). The tyrosine ratio at P60 was similar between LOW and MED ( $P = 0.107$ ), LOW and HIGH ( $P = 0.070$ ) and MED and HIGH ( $P = 0.552$ ) but was significantly higher in MED and HIGH at PTT compared to LOW and PLA ( $P < 0.05$ ). There was no difference between MED and HIGH at PTT ( $P = 0.641$ ). The tyrosine ratio remained unchanged in PLA ( $P = 0.433$ ). The tyrosine ratio increased 4.8 fold from baseline in LOW, 7.3 fold in MED and 7.3 fold in HIGH. Individual amino acid data are presented in table 3. Plasma tyrosine concentration was similar at rest in all trials ( $P = 0.978$ ). Tyrosine concentration increased significantly from rest to P60 in all tyrosine conditions ( $P < 0.01$ ) and then remained elevated between P60 and PTT ( $P = 0.745$ ). Tyrosine concentration was significantly different between PLA and the LOW, MED and HIGH tyrosine doses ( $P < 0.05$ ). Tyrosine concentration was significantly different between LOW and HIGH ( $P = 0.035$ ) although there was no difference between LOW and MED ( $P = 0.198$ ) or MED and HIGH ( $P = 0.792$ ). Amino acids displayed no significant interaction across trials ( $P = 0.471$ ). Valine ( $P = 0.360$ ), leucine ( $P = 0.253$ ) and isoleucine ( $P = 0.183$ ) concentrations were similar across all trials although did decrease over time in all trials ( $P < 0.05$ ). Valine decreased significantly from rest to PTT in PLA ( $P = 0.007$ ), ( $P = 0.015$ ) in LOW and ( $P = 0.00$ ) in MED. In HIGH, valine increased significantly between rest and P60 ( $P = 0.005$ ) although decreased significantly overall between rest and PTT ( $P = 0.001$ ). Leucine decreased significantly from rest to PTT in PLA ( $P = 0.00$ ), ( $P = 0.004$ ) in LOW and ( $P = 0.001$ ) in MED. In HIGH, leucine increased significantly between rest and P60 ( $P = 0.001$ ) but decreased significantly overall between rest and PTT ( $P =$

0.00). Isoleucine decreased significantly from rest to PTT in PLA ( $P = 0.00$ ), ( $P = 0.009$ ) in LOW and ( $P = 0.002$ ) in MED. In HIGH, isoleucine increased from rest to P60 ( $P = 0.001$ ) but decreased significantly overall between rest and PTT ( $P = 0.004$ ). The plasma ratio of free (f) tryptophan:  $\Sigma$ LNAA was similar at rest between all trials ( $P = 0.141$ ) although did decrease significantly over time in LOW ( $P = 0.02$ ), MED ( $P = 0.00$ ) and HIGH ( $P = 0.00$ ). In PLA, the tryptophan:  $\Sigma$ LNAA ratio decreased from baseline to PTT ( $P = 0.36$ ) although the ratio at all time points was significantly different to the tyrosine doses ( $P < 0.05$ ). There were no significant differences in the f-tryptophan:  $\Sigma$ LNAA ratio between the tyrosine doses ( $P > 0.05$ ). f-tryptophan concentration was similar in all trials ( $P = 0.846$ ) but did change over time in PLA and HIGH ( $P < 0.05$ ). Methionine and phenylalanine remained unchanged from rest across all trials ( $P > 0.05$ ). Decreased concentration in some amino acids comparative to increased tyrosine concentration in the tyrosine trials demonstrates the increase in the tyrosine: LNAA ratio was a result of these changes. Specifically, overall decreases from rest to PTT in; valine, leucine and isoleucine in all tyrosine trials & f-tryptophan in the  $400 \text{ mg} \cdot \text{kg body mass}^{-1}$  trial led to the change in the tyrosine: LNAA ratio.

Blood glucose concentration was similar between all trials ( $P = 0.623$ ) although was affected by time ( $P = 0.002$ ). Blood glucose decreased significantly from REST to PRE in all conditions ( $P < 0.05$ ). Blood glucose rose significantly in LOW between PRE and P60 ( $P = 0.004$ ). In HIGH, blood glucose rose significantly between PRE and P60 ( $P = 0.039$ ) and then decreased significantly between P60 and PTT ( $P = 0.004$ ). From rest to the end of the time trial, blood glucose decreased from  $4.5 \pm 0.2$  to  $4.3 \pm 0.3 \text{ mmol} \cdot \text{L}^{-1}$  in PLA;  $4.4 \pm 0.3$  to  $4.3 \pm 0.4 \text{ mmol} \cdot \text{L}^{-1}$  in LOW;  $4.6 \pm 0.2$  to  $4.2 \pm 0.2 \text{ mmol} \cdot \text{L}^{-1}$  in MED and  $4.5 \pm 0.3$  to  $4.1 \pm 0.5 \text{ mmol} \cdot \text{L}^{-1}$  in HIGH. No significant differences were found in blood lactate concentrations between trials ( $P = 0.822$ ) but were significantly increased over time in all conditions ( $P < 0.01$ ). Significant increases in

blood lactate were identified between PRE and Post 60 ( $P = 0.038$ ) and between Post 60 and PTT ( $P = 0.009$ ). From rest to the end of the time trial, blood lactate increased from  $0.6 \pm 0.1$  to  $3.3 \pm 1.6 \text{ mmol}\cdot\text{L}^{-1}$  in PLA;  $0.7 \pm 0.3$  to  $2.9 \pm 1.8 \text{ mmol}\cdot\text{L}^{-1}$  in LOW;  $0.6 \pm 0.17$  to  $3.2 \pm 1.3 \text{ mmol}$  in MED $\cdot\text{L}^{-1}$  and  $0.6 \pm 0.19$  to  $3.1 \pm 1.4 \text{ mmol}\cdot\text{L}^{-1}$  in HIGH.

Plasma volume decreased in a similar fashion following drinks ingestion across all trials ( $P = 0.287$ ). From rest to PRE, plasma volume decreased by  $2.8 \pm 4.6 \%$  in PLA;  $1.6 \pm 2.1 \%$  in LOW;  $3.0 \pm 1.4 \%$  in MED and  $1.0 \pm 2.4\%$  in HIGH. Between PRE and Post 60, plasma volume continued to decrease by  $6.6 \pm 5.4 \%$  in PLA;  $5.8 \pm 2.3 \%$  in LOW;  $6.0 \pm 3.1 \%$  in MED and  $1.2 \pm 8.3 \%$  in HIGH ( $P = 0.76$ ). Plasma volume between Post 60 to PTT demonstrated a continued decrease from rest by  $9.4 \pm 5.5 \%$  in PLA;  $7.5 \pm 3.7 \%$  in LOW;  $9.8 \pm 2.9 \%$  in MED and  $9.3 \pm 5.4 \%$  in HIGH ( $P = 0.001$ ).



**Figure 2. Change in ratio of plasma tyrosine:  $\Sigma$ LNAA (sum of the plasma concentration of valine, leucine, isoleucine, methionine, phenylalanine and tryptophan) in the placebo trial (PLA), 150 mg·kg (LOW), 300 mg·kg (MED) and 400 mg·kg (HIGH) tyrosine trials. (n = 8). \* Significant difference between the tyrosine trials and placebo trial ( $P < 0.05$ ).**

	PLA				LOW				MED				HIGH			
Amino acids (μmol·L <sup>-1</sup> )	REST	PRE	P60	PTT	REST	PRE	P60	PTT	REST	PRE	P60	PTT	REST	PRE	P60	PTT
Tyrosine	115 (± 17)	100 (±21)	119 (±19)	119 (±11)	113 (±24)	434*,** (±155)	509*,** (±179)	436*,** (±148)	111 (±17)	449* ** (±127)	677*, ** (±212)	686*,**, (±152)	115 (±26)	451*, ** (±110)	651*, ** (±148)	739*,**, # (±166)
Valine	354 (±79)	351 (±64)	346* (±57)	311* (±77)	364 (±78)	399 (±89)	340 (±58)	281* (±70)	345 (±44)	336* (±47)	324* (±42)	298* (±39)	360 (±67)	326 (±59)	417* (±67)	298* (±48)
Leucine	192 (±51)	184 (±36)	159* (±26)	138* (±29)	183 (±52)	180 (±64)	152 (±37)	125* (±37)	187 (±35)	164* (±36)	143* (±28)	120* (±19)	212 (±43)	161* (±30)	233* (±40)	134* (±15)
Isoleucine	81 (±21)	79* (±15)	71* (±12)	64* (±13)	79 (±26)	77 (±26)	63 (±13)	60* (±14)	82 (±18)	69* (±17)	62* (±11)	58* (±9)	81 (±22)	67* (±16)	104* (±20)	59* (±8)
Methionine	24 (±2)	24 (±2)	27 (±3)	27 (±3)	27 (±5)	27 (±4)	28 (±3)	26 (±4)	24 (±4)	24 (±4)	25 (±4)	26 (±4)	26 (±5)	25 (±4)	26 (±4)	25 (±4)

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<b>Phenylalanine</b>	<b>74</b> (±8)	<b>73</b> (±8)	<b>86</b> (±8)	<b>86</b> (±8)	<b>71</b> (±14)	<b>76</b> (±12)	<b>82</b> (±15)	<b>80</b> (±21)	<b>67</b> (±10)	<b>73</b> (±19)	<b>77</b> (±13)	<b>84</b> (±20)	<b>74</b> (±6)	<b>74</b> (±5)	<b>71</b> (±9)	<b>88</b> (±18)
<b>Tryptophan</b>	<b>121</b> (±5)	<b>127</b> (±14)	<b>113</b> (±9)	<b>95*</b> (±15)	<b>122</b> (±22)	<b>128</b> (±20)	<b>120</b> (±23)	<b>103</b> (±25)	<b>115</b> (±16)	<b>121</b> (±16)	<b>115</b> (±10)	<b>99</b> (±10)	<b>130</b> (±21)	<b>135</b> (±21)	<b>113*</b> (±19)	<b>95*</b> (±24 )

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Table 3. Plasma amino acid concentrations

**Values represented as mean (± SD)**

**\* Significantly different to REST time point in same trial (P < 0.05)**

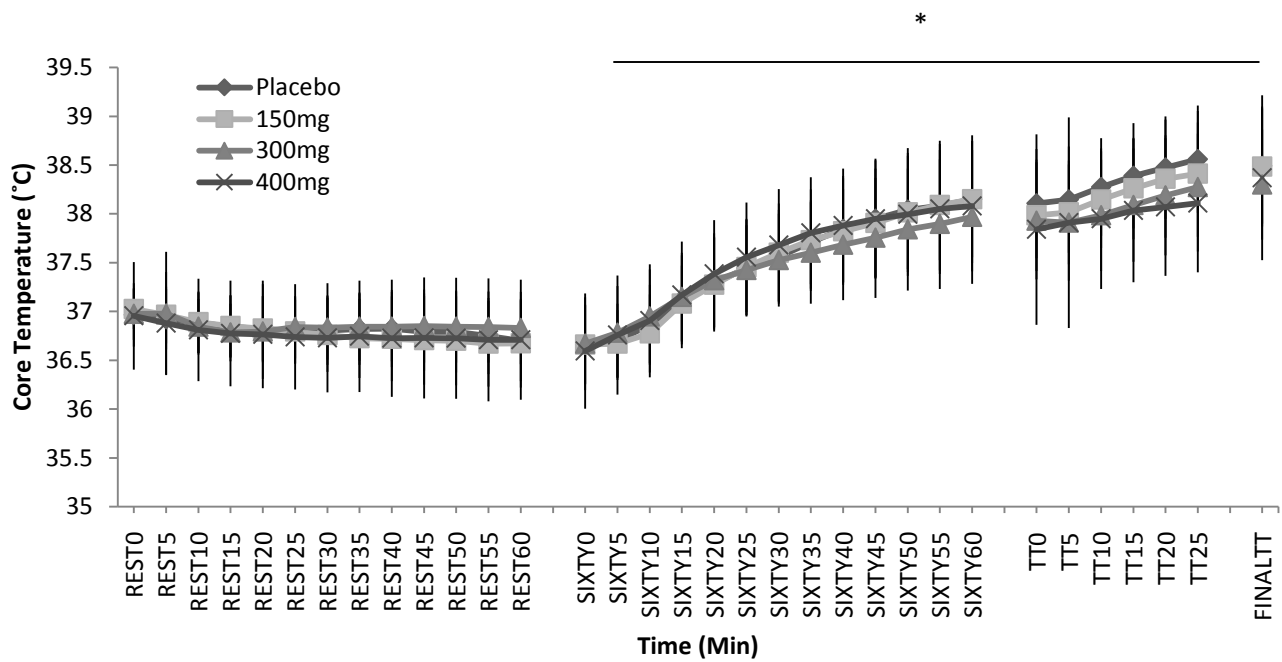
**\*\* Significantly different to PLA trial at same time point (P < 0.05)**

**# Significantly different to LOW trial at same time point (P < 0.05)**

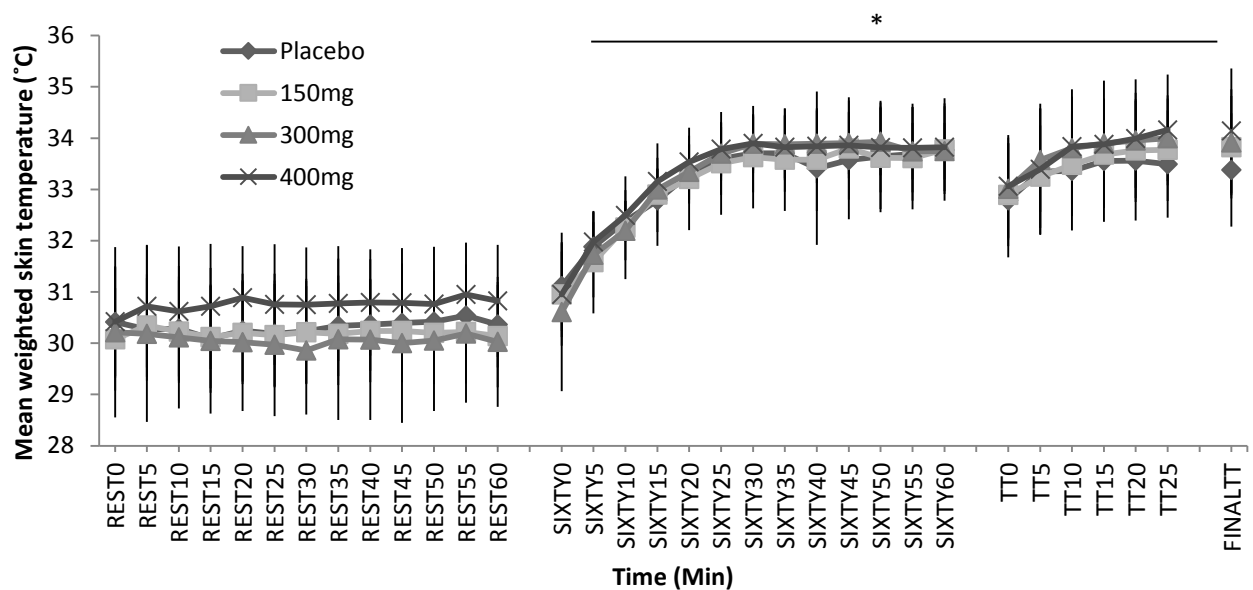


## Temperature Measurements

Friedmans test indicated a gradual increase in core temperature from the start of the 60 minutes exercise period to the end of the time trial ( $P < 0.05$ ) across all trials (Figure 3). There were no significant differences in  $T_{core}$  between trials during the rest period ( $P = 0.326$ ) or throughout exercise ( $P > 0.05$ ). At the end of the sixty minute exercise period, median  $T_{core}$  was 38.15 (1.31) °C in PLA; 38.15 (1.19) °C in LOW; 37.97 (1.37) °C in MED and 38.08 (1.34) °C in HIGH. At the end of the time trial, median core temperature was 38.49 (1.18) °C in PLA; 38.48 (1.23) °C in LOW; 38.30 (1.13) °C in MED and 38.37 (1.69) °C in HIGH. Due to technical issues mean weighted skin temperature data was available for only five subjects and is presented as such. There were no significant differences in  $T_{skin}$  between trials during the rest period ( $P = 0.115$ ). Drink ingestion had no effect on skin temperature across trials during exercise ( $P = 0.127$ ) although skin temperature did increase significantly from rest to the end of the time trial in all trials ( $P < 0.05$ ), plateauing after 30 minutes of submaximal, constant intensity exercise. Mean weighted skin temperature at the end of the 60 minute period of exercise was  $33.76 \pm 0.85$  °C in PLA;  $33.77 \pm 0.91$  °C in LOW;  $33.74 \pm 0.55$  °C in MED and  $33.82 \pm 0.85$  °C in HIGH. Skin temperature declined briefly, between the end of the 60 minute submaximal period of exercise before increasing again at the start of the time trial across all trials ( $P < 0.05$ ). Skin temperature at the end of the time trial increased significantly from the start of the time trial in the LOW, MED and HIGH tyrosine trials ( $P < 0.05$ ). Skin temperature increased in the PLA trial but the increase was not significant ( $P = 0.105$ ). Mean weighted skin temperature at the end of the time trial was  $33.38 \pm 1.10$  °C in PLA;  $33.82 \pm 1.41$  °C in LOW;  $33.91 \pm 1.03$  °C in MED and  $34.14 \pm 1.21$  °C in HIGH (Figure 4.).



**Figure 3. Median (range) core temperature during the 1 hour rest period, 60 minutes of submaximal, constant-load exercise and a simulated time trial. Time trial data presented up to 25 mins (last common time-point when all participants were still exercising) and at end of exercise, in the placebo trial (PLA), 150 mg·kg (LOW), 300 mg·kg (MED) and 400 mg·kg (HIGH) tyrosine trials. (n = 8). \* Significantly different from SIXTY0 in all trials ( $P < 0.05$ )**

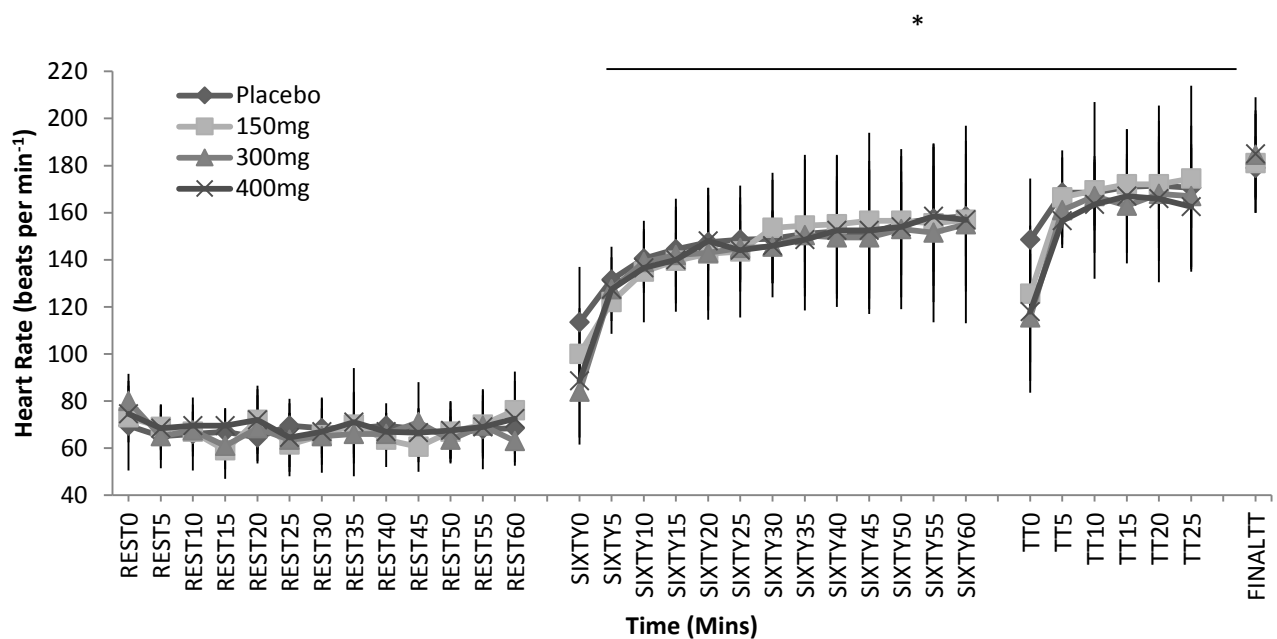


**Figure 4.** Mean ( $\pm$  SD) weighted skin temperature during the 1 hour rest period, 60 minutes of submaximal, constant load exercise and a simulated time trial. Time trial data presented up to 25 mins (last common time-point when all participants were still exercising) and at end of exercise, in the placebo trial (PLA), 150 mg·kg (LOW), 300 mg·kg (MED) and 400 mg·kg (HIGH) tyrosine trials. (n = 5) \* Significantly different from SIXTY0 in all trials ( $P < 0.05$ )

### Heart Rate:

Friedmans test indicated that heart rate increased over time from the start of the 60 minute exercise period to the end of the time trial ( $P < 0.05$ ) although there were no significant differences in heart rate between trials at any point ( $P > 0.05$ ) (Figure 5). Heart rate transiently decreased at the end of the 60 minute period of exercise prior to the start of the time trial ( $P < 0.01$ ). At the end of the 60 minute exercise period median heart rate was 158 (64) beats·min<sup>-1</sup> in

PLA; 157 (56) beats·min<sup>-1</sup> in LOW; 155 (84) beats·min<sup>-1</sup> in MED and 157 (31) beats·min<sup>-1</sup> in HIGH. At the end of the time trial, median heart rate was 179 (27) beats·min<sup>-1</sup> in PLA; 181 (42) beats·min<sup>-1</sup> in LOW; 184 (49) beats·min<sup>-1</sup> in MED and 185 (37) beats·min<sup>-1</sup> in HIGH. This represented 98 ± 6 %, 97 ± 7 %, 98 ± 8 % and 96 ± 7 % of maximum heart rate in PLA, LOW, MED and HIGH respectively.



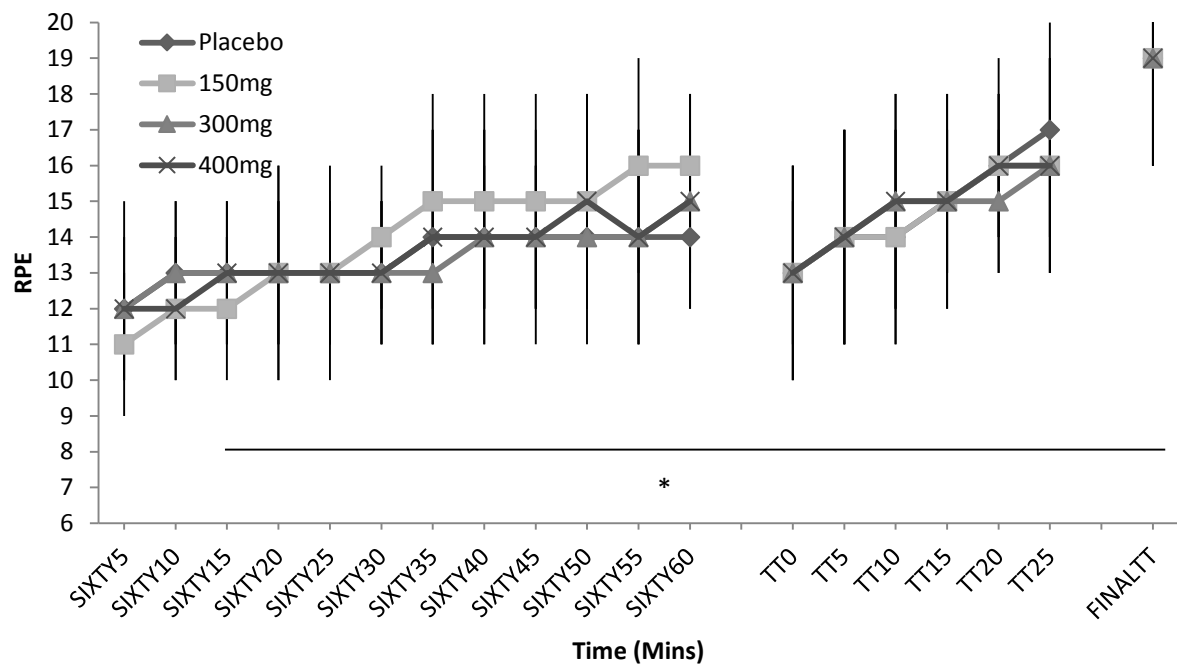
**Figure 5. Median (range) heart rate during the 1 hour rest period, 60 minutes of submaximal, constant load exercise and a simulated time trial. Time trial data presented up to 25 mins (last common time-point when all participants were still exercising) and at end of exercise, in the placebo trial (PLA), 150 mg·kg (LOW), 300 mg·kg (MED) and 400 mg·kg (HIGH) tyrosine trials. (n=8) \* Significantly different from SIXTY0 in all trials (P < 0.05)**

## Fluid Intake and Body Mass Losses

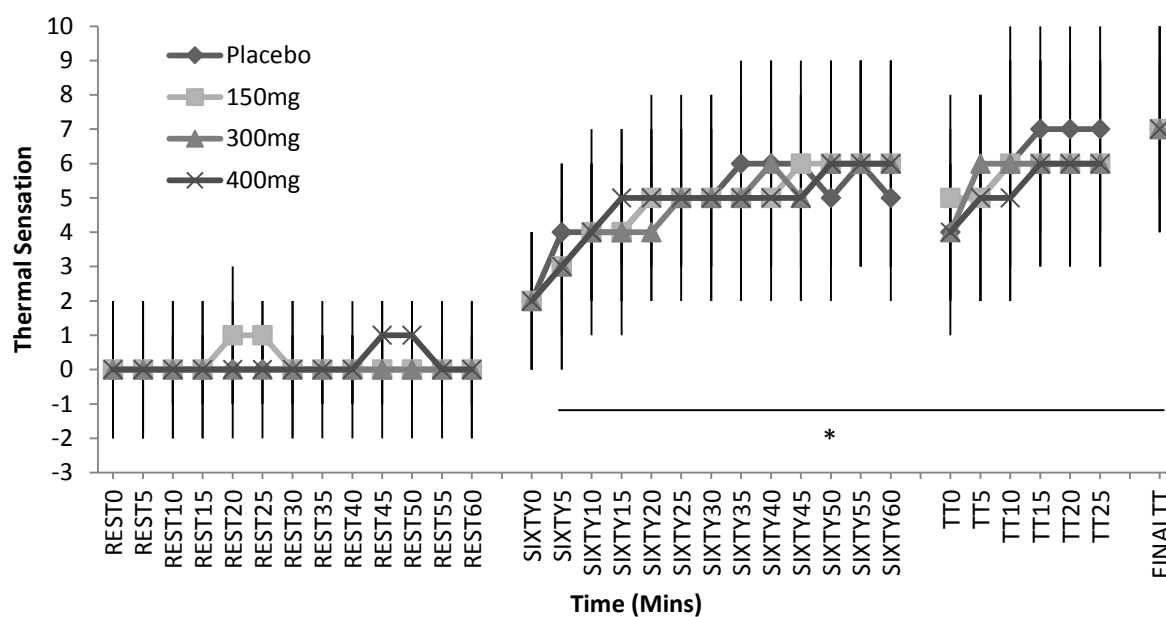
Participants consumed similar volumes of fluid in each trial ( $P = 0.654$ ;  $221.3 \pm 209.8$  mL in PLA;  $241.75 \pm 154.1$  mL in LOW;  $215.6 \pm 174.4$  mL in MED and  $300.75 \pm 237.5$  mL in HIGH). Body mass losses, calculated as the difference between pre and post body mass, and accounting for fluid intake and urine output (collected out-with exercise), was similar across all trials ( $P = 0.193$ ;  $0.4 \pm 0.7$  kg in PLA;  $0.8 \pm 0.6$  kg in LOW;  $0.5 \pm 0.3$  kg in MED and  $0.5 \pm 0.4$  kg in HIGH). Expressed as a percentage change, body mass losses were similar across trials ( $P = 0.495$ ;  $0.01 \pm 0.006$  % in PLA;  $0.01 \pm 0.007$  % in LOW;  $0.01 \pm 0.006$  % in MED and  $0.01 \pm 0.006$  % in HIGH). Sweat loss was also similar across all trials ( $P = 0.606$ ;  $603 \pm 477$  mL in PLA;  $808 \pm 648$  mL in LOW;  $646 \pm 611$  mL in MED and  $524 \pm 724$  mL in HIGH).

## Subjective Ratings

Friedmans test demonstrated that RPE increased gradually from start of exercise in all trials ( $P < 0.05$ ) although did not differ between trials ( $P > 0.05$ ) (Figure 6). Median RPE at the end of the 60 minute period of submaximal exercise was 14 (5) arbitrary units in PLA; 16 (4) in LOW; 15 (5) in MED and 15 (4) in HIGH. Median RPE at the end of the time trial was 19 (6) in PLA; 19 (5) in LOW; 19 (5) in MED and 19 (6) in HIGH. Thermal sensation increased gradually from the start of exercise ( $P < 0.05$ ) although no differences in thermal sensation were found between trials ( $P > 0.05$ ) (Figure 7). Median thermal sensation at the end of the 60 minute period of exercise was 5 (6) arbitrary units in PLA; 6 (5) in LOW; 6 (5) in MED and 6 (6) in HIGH. A rating between 5 and 6 represents a rating of “Very hot, uncomfortable” on the thermal sensation scale. Thermal sensation values at the end of the time trial were similar across all trials ( $P = 0.777$ ) with median value of 7 (7) arbitrary units in PLA; 7 (5) in LOW; 7 (5) in MED and 7 (5) in HIGH. Thermal sensation at the end of the time trial represented ratings between “Very hot, uncomfortable” and “Extremely hot, close to limit”.



**Figure 6. Median (range) rating of perceived exertion values during the 60 minutes of submaximal constant load exercise and a simulated time trial. Time trial data presented up to 25 mins (last common time-point when all participants were still exercising) and at end of exercise, in the placebo trial (PLA), 150 mg·kg (LOW), 300 mg·kg (MED) and 400 mg·kg (HIGH) tyrosine trials (n=8). \* Significant increase from SIXTY10 in all trials (P < 0.05)**



**Figure 7. Median (range) thermal sensation values during the 60 minutes of submaximal constant load exercise and a simulated time trial. Time trial data presented up to 25 mins (last common time-point when all participants were still exercising) and at end of exercise, in the placebo trial (PLA), 150 mg·kg (LOW), 300 mg·kg (MED) and 400 mg·kg (HIGH) tyrosine trials (n=8). \* Significant increase from SIXTY0 in all trials (P < 0.05)**



## Discussion

The present study hypothesized that administration of tyrosine in the medium and high dose would enhance exercise performance in the heat based upon tyrosine's role as a nutritional catecholamine precursor. Despite a firm theoretical basis for the use of tyrosine during exercise in the heat, the present study demonstrated that neither the low, medium or high tyrosine dose had an effect on exercise performance in the heat, compared to a placebo containing no tyrosine.

Previous studies have examined the effect of a single tyrosine dose on the ability to sustain exercise in warm environmental conditions, producing mixed results to date (Tumilty *et al.*, 2011; Watson *et al.*, 2012; Tumilty *et al.*, 2014; Coull *et al.*, 2015). This is the first study to examine the effect of three different tyrosine doses (low, medium and high) on exercise performance in the heat. Exercise in the heat should create the stressful environment necessary to increase the firing rates of dopaminergic neurons and increase catecholamine synthesis (Powers, Howley and Cox, 1982), resulting in a decrease in brain dopamine and precursor availability (Fernstrom, 2003; Melamed *et al.*, 1980). Studies by Tumilty *et al.*, (2011) and Tumilty *et al.*, (2013) suggest an association between tyrosine availability and exercise tolerance when exercising in the heat. Previous studies in animals have demonstrated that increasing the ratio of a particular amino acid to its competitors can increase brain uptake (Fernstrom and Wurtman, 1972; Fernstrom and Faller, 1978) while others have demonstrated that increased precursor uptake into the brain can result in increased turnover of that precursor's neurotransmitter (Brady *et al.*, 1980; Acworth *et al.*, 1988). Additional availability of tyrosine, via supplementation, should increase transport into the brain and should therefore allow the continued synthesis of dopamine during exposure to exercise in the heat. Tumilty *et al.*, (2011) demonstrated tyrosine to have a beneficial effect on exercise in warm conditions having reported significant increase in

the plasma ratio of tyrosine: competing amino acids, in comparison to a placebo, when exercising in the heat. The high and medium dose of tyrosine in the present study was hypothesised to have the greatest performance benefit as it would result in the highest increase in the plasma ratio of tyrosine: competing amino acids compared to the placebo and low dose, thus transporting more into the brain for dopamine synthesis. This hypothesis is supported by the plasma amino acid data after tyrosine was administered. The data demonstrate that all three doses of tyrosine increased the plasma ratio of tyrosine: competing amino acids significantly in comparison to the placebo trial, with the medium and high dose (7.3 fold) increasing the ratio significantly more compared to the low dose (4.8 fold). The data does however demonstrate an important finding that the medium dose of tyrosine increased the plasma tyrosine ratio to the same extent as the high dose, therefore acutely supplementing tyrosine in a dose higher than 300 mg·kg body mass<sup>-1</sup> may be unnecessary.

The importance of dopamine availability during exercise is highlighted by Davis and Bailey (1997) who suggest a high ratio of brain dopamine: 5-HT will maintain levels of motivation, arousal and drive and subsequently allow exercise to continue. Conversely a high ratio of brain 5-HT: dopamine is associated with reduced arousal and fatigue (Davis and Bailey, 1997). This demonstrates the potential importance of supplying additional tyrosine as a precursor to dopamine synthesis, to maintain a high ratio of brain dopamine: 5-HT and thus, prolong exercise. Evidence from rat studies demonstrates that fatigue is associated with a reduction in brain dopamine synthesis whereas fatigue is delayed when dopamine synthesis and metabolism is maintained. Chaouloff *et al.*, (1987) demonstrated that the provision of amphetamines increased dopamine activity and potentiated the inhibition of serotonin during exercise in rats. This is supported by Yeghiayan *et al.*, (2001) which found, in rats, amphetamine improved forced swim test performance following hypothermia. Amphetamine administration is also associated with

improved knee extension strength, acceleration, anaerobic capacity and time to exhaustion in humans (Chandler and Blair, 1980). Amphetamines have potent effects on brain dopamine release. The mechanisms of amphetamine works similarly to reuptake inhibitors but differently to tyrosine in that amphetamine administration increases the release of, and prevents the reuptake of, catecholamines at receptor sites (Cooper, 2003). Tyrosine however is tightly regulated by tyrosine hydroxylase and may only become effective under stressful conditions (During *et al.*, 1988; Melamed *et al.*, 1980). In conditions where dopaminergic neurons are not suitably stressed, additional tyrosine availability is likely to be affected by end-product inhibition of tyrosine hydroxylase (Badaway and Williams, 1982). The differences in the mechanistic actions of tyrosine and other drugs on dopamine synthesis may explain different findings. Cordery, James, Peirce, Maughan and Watson (2016) propose that dopamine release is determined by either the cytosolic or the vesicular dopamine pool. L-DOPA induced increases in dopamine release are reliant on the vesicular dopamine pool via synaptic vesicle exocytosis and phasic signalling (Cordery *et al.*, 2016). The authors suggest that the two pools of dopamine available have a role in distinguishing drug actions and individual susceptibility to the effects of particular drugs (Cordery *et al.*, 2016). The differences in the effects of phasic and tonic firing of dopamine release may explain why catecholamine precursors, such as tyrosine, affect exercise performance in a less consistent way in comparison to other drugs such as reuptake inhibitors. Evidence from previous studies demonstrates that dopamine reuptake inhibition increases tonic stimulation of low affinity postsynaptic receptors and results in desensitization of phasic dopamine signals (Dreyer and Hounsgaard, 2013). Tonic dopamine signalling regulates extracellular dopamine and is associated with “average reward” of behaviour, therefore when dopamine reuptake inhibitors are administered; this can increase motivation and “average reward” which may result in an improved performance. The relative success of reuptake inhibitors in improving exercise performance (Bridge *et al.*, 2003; Watson *et al.*, 2005) demonstrates this point further. It is

possible in the present study that tyrosine administration increased phasic signalling and may explain a lack of improvement in exercise performance with tyrosine intake although at present this is purely speculative. Despite a sound neurochemical basis for the use of tyrosine as a precursor to dopamine in the current study, no performance benefit was found. Regardless of the increased availability of tyrosine, it is possible that dopamine synthesis did not increase as the nature of the exercise was not appropriate (i.e. not stressful enough) to increase the rate of neuronal firing and subsequently deplete tyrosine within brain. As a result, the increase in tyrosine availability, as demonstrated by the plasma amino acid data, was likely subject to end product inhibition of tyrosine hydroxylase.

Exercise in the heat was assumed to be suitably demanding to stress central catecholamines and increase the demand for tyrosine in the brain. Bridge *et al.*, (2003) and Watson *et al.*, (2005) highlighted the importance of brain dopamine activity during exercise in warm conditions. Bridge *et al.*, (2003) administered a dual 5-HT receptor agonist and a dopamine receptor agonist (buspirone) or buspirone and pindolol (a 5-HT receptor antagonist) on two separate occasions before exercising to exhaustion in the heat. Results demonstrated that high activity of the dopaminergic pathways in the hypothalamus are a predictor of the ability to exercise in the heat (Bridge *et al.*, 2003). Furthermore, Watson *et al.*, (2005) administered either placebo or bupropion, a dual dopamine/noradrenaline reuptake inhibitor, over four trials prior to exercising in both a temperate and warm environment. This study demonstrated that bupropion improved time trial performance in the heat but not in a temperate environment. It was demonstrated that bupropion, as a dual dopamine/noradrenaline reuptake inhibitor, enhanced exercise performance in the heat and allowed subjects to exercise at higher core temperatures when compared to temperate exercise where no such effect was recorded. This work demonstrates there to be a specific demand for dopamine in the heat which is not present during temperate conditions and

should provide the environment necessary to upregulate brain dopamine neurons. Additional tyrosine may then result in an increase in brain uptake, potentially maintaining brain dopamine synthesis (Tumilty *et al.*, 2011). Studies by During, Acworth and Wurtman (1989) and Murrin, Morgenroth and Roth (1976) have shown that when animals are exposed to stressful treatments, such as drug administration to accelerate nigrostriatal firing or electrical stimulation of the medial forebrain, brain neurotransmitter turnover increases and tyrosine becomes depleted in some neuronal populations. This causes an increased affinity between tyrosine hydroxylase and tyrosine which increases dopamine synthesis to maintain dopamine synthesis (Roth, Walters and Morgenroth, 1974). In rats, tyrosine administration has been shown to successfully maintain catecholamine synthesis and ameliorate behavioural deficits during exposure to tail shock (Reinstein *et al.*, 1984), heat stress (Lieberman *et al.*, 2005) and cold swim stress (Brady *et al.*, 1980). While studies involving cognitive function and tyrosine have produced promising results, previous work examining acute tyrosine administration and exercise in the heat in humans have been mixed. Furthermore, exercise and tyrosine administration in temperate conditions have failed to find any beneficial effect. Watson *et al.*, (2012) suggests an increase in the blood ratio of tyrosine: competing amino acids will only prove effective in increasing catecholamine synthesis during periods of increased stress over and above that of most exercise conditions, a condition not considered to have been achieved in their work. Similarly, Coull *et al.*, (2015) found no significant difference between an acute dose of 150 mg·kg body mass<sup>-1</sup> tyrosine or placebo on time trial load carriage performance test in the heat, despite a marked increase in serum tyrosine concentration. Tumilty *et al.*, (2014) found tyrosine to have no performance benefit on exercise performance in the heat when a dose of 150 mg·kg body mass<sup>-1</sup> was administered prior to exercise despite significant increases in the tyrosine ratio to competing amino acids. However, an earlier study by the same authors demonstrated that tyrosine had a beneficial effect on exercise capacity in the heat. This study demonstrated that a 150 mg· kg

body mass<sup>-1</sup> dose of tyrosine increased the ratio of tyrosine to competing amino acids and subsequently improved exercise capacity in the heat by  $15 \pm 11$  % in comparison to a placebo. To date, this is the only study to show a benefit of acute tyrosine supplementation on exercise in the heat. It is possible that the combination of heat and a performance time trial used in the present study was not demanding enough to increase neuronal firing and elicit changes in catecholamine metabolism. Therefore, any additional tyrosine availability would have saturated tyrosine hydroxylase resulting in end product inhibition (Badawy and Williams, 1982).

When comparing the results of the present study and other studies examining acute tyrosine supplementation, there is currently insufficient evidence available to recommend tyrosine prior to performing physical exercise in physically stressful and/ or demanding conditions. In comparison, tyrosine's effect on cognition has been shown to be beneficial in a number of studies. Evidence suggests tyrosine has a beneficial effect on maintaining cognitive function during stress exposure. Tyrosine administration has been shown to improve cognitive function during exposure to exercise heat stress (Coull *et al.*, 2014) and improve memory during extreme psychological and physical stress (Deijen *et al.*, 1999; Mahoney *et al.*, 2007). Liebermann *et al.*, (2014) demonstrated tyrosine to increase feelings of anger in response to acute stress which consisted of two mock interrogations during several days of simulated captivity. It was suggested that the response of anger was an adaptive emotional response to stressful environments, which was enhanced with tyrosine. However the success of tyrosine administration on cognitive control may be dependent on individual dopamine function (Jongkees, Hommel and Colzato, 2014). The authors suggest individual differences exist in dopamine function, with some carrying the allele associated with low prefrontal dopamine function. Individuals with this allele may particularly benefit from increased tyrosine availability (e.g. via supplementation) as this may assist in

increased conversion of tyrosine to dopamine in the brain. This is a possibility which may be considered when designing future research studies involving tyrosine.

It is apparent from the power output values that even pacing was adopted throughout each trial, regardless of the amount of tyrosine administered prior to exercise. It was hypothesised that tyrosine administration in the medium and high dose would result in faster time trial performance, and therefore higher power output during the time trial, in comparison to the low dose and placebo. It is obvious that if a pacing strategy was employed by participants during the time trial, this was unaffected by tyrosine. St Clair-Gibson *et al.*, (2006) states that the pacing of a particular event is influenced by the knowledge of where the endpoint of the activity lies. The authors also state that the brain interprets several factors which include the memories of previous events of similar distance or duration as well as external factors. These include environmental conditions and metabolic factors which together, form an optimal pacing strategy for the individual which is necessary in order to avoid catastrophic failure of the task. This may offer an explanation as to why Tumilty *et al.*, (2011) found a beneficial effect of tyrosine in a capacity trial but found no significant difference in acute tyrosine supplementation when participants completed a performance trial (Tumilty *et al.*, 2014). This may demonstrate that tyrosine administration is most effective during constant load, submaximal exercise when individualised pre-determined power outputs are provided which may be perceived as more physically demanding than a self-paced time trial and therefore subjectively more demanding (Tumilty, 2011). This is supported in work by Lander, Butterly and Edwards (2009) which demonstrated that a fixed paced 5000 m time trial on a rowing ergometer resulted in increased levels of blood lactate, attainment of a higher core temperature and higher RPE in comparison to self-paced trial over the same distance. These differences were observed despite the time taken to complete the time trial and power outputs remaining similar throughout both trial conditions. However,

Watson *et al.*, (2012) found no performance benefit from the same dose of tyrosine on exercise capacity during a similar protocol with the authors suggesting that the exercise demands were insufficient to recognise any performance benefit. It is plausible to suggest that the exercise protocol used in the present study, where participants were free to self-pace the time trial, was not physiologically or psychologically demanding enough to elicit increased catecholamine metabolism and therefore the additional tyrosine availability was ineffective. Allowing participants to control their power output 'moment to moment' may not provide the environment necessary to considerably increase dopamine metabolism in comparison to a constant load power output which may be interpreted as more physically demanding (Lander, Butterly and Edwards, 2009). Perhaps a more physically demanding, or longer, exercise protocol may have demonstrated a greater effect of tyrosine. In future work, increasing the physical demand of the exercise protocol may be achieved through increasing individualised work target quantities during the time trial or working at a greater percentage of  $\dot{V}O_{2peak}$  during submaximal exercise.

Tyrosine, as a central catecholamine precursor, may act to increase central noradrenaline activity and result in reduced exercise performance (Tumilty *et al.*, 2014). This is supported by Roelands *et al.*, (2008) who demonstrated that acute administration of reboxetine, a norepinephrine reuptake inhibitor, reduced exercise capacity in cyclists in both normal and high ambient temperatures. It is thought this occurred because reboxetine exerted both peripheral and central effects on the body including accelerations in both resting and exercise heart rate upon administration. More than half of the participants also reported feeling cold only when reboxetine was administered which may have negatively affected exercise performance. While not statistically significant from any other dose, the present data demonstrate that time trial performance in the present study was slowest with the 400 mg·kg body mass<sup>-1</sup> dose while the 150 mg·kg body mass<sup>-1</sup> dose and 300 mg kg· body mass<sup>-1</sup> were also slower compared to placebo.



This may provide support for the explanation that additional tyrosine availability could have increased brain noradrenaline activity and therefore reduced exercise performance. Badawy and Williams (1982) demonstrated a low dose of tyrosine ( $20 \text{ mg} \cdot \text{kg body mass}^{-1}$ ) increased cerebral catecholamine synthesis whereas higher doses ( $50 \text{ mg} \cdot \text{kg body mass}^{-1}$  up to  $500 \text{ mg} \cdot \text{kg body mass}^{-1}$ ) were associated with reduced catecholamine levels attributed to feedback inhibition of tyrosine hydroxylase activity. Strüder *et al.*, (1998) reported increased plasma prolactin concentration as a result of oral tyrosine ingestion suggesting that tyrosine has the ability to enter the brain and act centrally. Given that increased dopamine release from the anterior pituitary usually inhibits the release of prolactin, this result was somewhat surprising. It is possible that the increased availability of brain tyrosine in the Strüder *et al.*, (1998) study may have caused feedback inhibition of tyrosine hydroxylase and reduced dopamine synthesis in the pituitary, increasing prolactin release.

The present results, and the results from other studies (Tumilty *et al.*, 2014; Coull *et al.*, 2015), may demonstrate that exercise in the heat is simply not demanding enough to elicit changes in brain catecholamine metabolism such that brain tyrosine availability becomes limiting to further catecholamine synthesis. If the stress on subjects was sufficient in these studies, neuronal firing would have been increased and catecholamine metabolism maintained. When tyrosine has been supplemented to humans in highly stressful situations, it has been shown to reduce deficits in cognitive function in studies involving cold exposure (Shurtleff *et al.*, 1994) and very intensive military combat training (Deijen *et al.*, 1999). Perhaps the very nature of the stress exposure, be it the magnitude, duration or realism of the stressor determines the subsequent effect tyrosine has on catecholamine metabolism. Prolonged exposure to stress may deplete catecholamines and increase reliance on tyrosine availability. This seems to be particularly evident in studies examining cognitive functions dependent upon brain dopamine function, but not as convincing in

exercise studies. It is suggested by Cordery *et al.*, (2016) that while fatigue and stress are reduced and other cognitive function improved in military based drills with tyrosine supplementation (Deijen *et al.*, 1999; Lieberman *et al.*, 2014), the same effects are not commonly found in laboratory based settings. This suggests that the psychological and physiological demands experienced by military personnel are more demanding compared to that which are experienced by participants in a controlled laboratory. This could explain why tyrosine is more effective during highly stressful or “real world” environments where the perception of stress is increased.

Similarities in both core temperature and skin temperature between trials demonstrate that tyrosine administration had little effect on thermoregulation. Research demonstrates that catecholamines may be involved in thermoregulation during exercise (Watson *et al.*, 2005; Hasegawa *et al.*, 2008). Watson *et al.*, (2005) demonstrated administration of a dopamine/noradrenaline reuptake inhibitor improved tolerance to high core temperatures during exercise in the heat. Despite different physiological mechanisms, the study demonstrates dopamine availability to have thermoregulatory effects with Hasegawa *et al.*, (2008) finding similar results in rats. Similarly, tyrosine had no effect on heart rate. These findings are generally in agreement with much of the literature concerned with exercise following acute tyrosine administration (Tumilty *et al.*, 2011; Watson *et al.*, 2012; Tumilty *et al.*, 2014; Coull *et al.*, 2015). Unsurprisingly, heart rate increased throughout exercise in the present study, which was likely due to cardiovascular strain, caused by heat exposure. This can detrimentally affect exercise capacity and performance when demands increase between the active muscles and the skin for increased blood flow, both of which require an increased cardiac output (Gonzalez-Alonso *et al.*, 2003). Performance can be severely affected when this, coupled with decreased stroke volume caused by dehydration occurs. However the present study (similarly to others; Tumilty *et al.*, 2011 and Tumilty *et al.*, 2014) ensured all participants were provided fluid

throughout exercise to avoid excessive dehydration. The urine osmolality data suggests this was effective. Significant increases in core temperature from rest demonstrate subjects were hyperthermic at the end of the 60 minute exercise period and at the end of the time trial. Morrison *et al.*, (2004) found increasing core temperature resulted in progressively reduced voluntary activation during an isometric maximal voluntary contraction despite only moderate cardiovascular strain. Increases in these measures appeared to be unaffected by tyrosine administration. This suggestion is supported by Tumilty, Davison, Beckmann and Thatcher (2014b) which found that administration of 150 mg·kg body mass<sup>-1</sup> tyrosine to have no effect on central fatigue, measured via peak and sustained handgrip maximal voluntary contraction force in hyperthermic subjects. Given that significant rises in both RPE and thermal sensation were present across all trials, it is possible to suggest that central fatigue developed as exercise progressed (Noakes, 2012; Nybo and Nielsen, 2001) which was likely due to hyperthermia. Considering dopamine's association with increased motivation, arousal, memory, reward mechanisms and increased attention (Meeusen *et al.*, 2006), it is perhaps surprising that RPE remained similar across all trials. This is despite administration of the medium and high dose of tyrosine which were both shown to significantly increase the tyrosine: LNAA ratio in comparison to placebo and the low tyrosine dose. Tumilty *et al.*, (2011) demonstrated that subjects exercised for significantly longer with 150 mg·kg body mass<sup>-1</sup> tyrosine despite similar RPE values compared to placebo, suggesting a possible increase in motivation with tyrosine administration. This may provide further evidence that the performance time trial was not sufficient to increase catecholamine metabolism and may suggest tyrosine to be more effective during an exercise capacity trial (Tumilty *et al.*, 2011) where effects on subjective measures and improved exercise tolerance have been demonstrated.

While the present study's protocol was replicated from previously published research, the study

contained some limitations which should be considered prior to any further research. As has been argued in depth in this discussion, the stress imposed on subjects during exercise may not have been substantial to elicit an effect on performance from tyrosine administration. The environmental conditions (30°C/60% relative humidity) may not have been demanding enough, therefore a future study examining tyrosine doses may benefit from exposing subjects to greater environmental stressors. Similarly, the laboratory environment may not be conducive to increasing dopamine synthesis with tyrosine administration and may be another limitation of the study. Given other previously discussed studies have found tyrosine to be effective in 'real world' environments, it may be beneficial to administer different tyrosine doses to individuals in professions of a more stressful nature (eg. military or hospital settings) to assess any performance benefits at different doses. The present work was also limited in that there was no measure of dopamine availability following tyrosine administration. As mentioned above, the presence (or lack of) of prolactin in the blood is an indicator of decreased/increased dopamine synthesis, respectively, and therefore may give a greater indication on the effectiveness of tyrosine administration during exercise.

In conclusion, the present study demonstrated that, contrary to the hypothesis, acute administration of the catecholamine precursor tyrosine in a low, medium or high dose, had no effect on simulated time trial performance in the heat, compared to a placebo containing no tyrosine. This is despite significant increases in the plasma ratio of tyrosine: amino acids which compete for brain uptake, when tyrosine was administered. The data does demonstrate that the medium dose of tyrosine increased the plasma tyrosine ratio to the same extent as the high dose, therefore acutely supplementing tyrosine in a dose higher than 300 mg·kg body mass<sup>-1</sup> may be unnecessary. Despite increases in the tyrosine ratio, it is likely that the exercise protocol and environmental conditions were not suitably demanding to increase brain catecholamine

metabolism to the extent that brain tyrosine availability became limiting to further catecholamine metabolism. The increased availability of tyrosine may have resulted in feedback inhibition of tyrosine hydroxylase within brain neurons. The main finding of this study is in agreement with much of the published literature which has examined acute tyrosine administration and exercise, despite a sound neurochemical basis for its use. At present, there is insufficient evidence available to recommend that acute oral tyrosine administration is capable of improving exercise performance in the heat.

## **Appendix**

**ABERYSTWYTH UNIVERSITY**  
**IBERS**  
**SPORT AND EXERCISE SCIENCE**  
  
**PARTICIPANT INFORMATION**

**The effect of varied tyrosine doses on exercise performance in the heat**

**Introduction**

Evidence suggests that fatigue during prolonged exercise in the heat may involve factors within the brain. In particular, alterations in chemicals found in the brain may result in increased lethargy and sleepiness, which may contribute to fatigue. Some of these brain chemicals are formed by substances called amino acids, and can be altered by the foods we eat. Consumption of the amino acid tyrosine has been shown to increase brain levels of some of these chemicals, specifically, dopamine and noradrenaline. This is particularly relevant because some studies suggest that changes in brain dopamine may influence exercise ability in the heat, potentially resulting in an improved exercise performance. The majority of previous studies involving tyrosine supplementation and exercise in the heat have used the same dose level with some showing improved exercise tolerance and others showing no effect. We want to determine whether there is an optimal tyrosine dose which enhances exercise in the heat by supplementing three different doses of tyrosine.

**What is required of me if I agree to take part in the study?**

You will be asked to visit the laboratory on six separate occasions, following an overnight fast. We will ask you to drink a pint (or 500 ml) of ordinary tap water two hours before visiting the laboratory to ensure that you are properly hydrated.

During the first visit, we will determine your maximal oxygen uptake value ( $\dot{V}O_{2max}$ ), which we will use to calculate the appropriate exercise intensity for subsequent visits. This is a maximal test where you will be asked to cycle at a fixed rev rate until you fatigue, while the workload gets progressively harder, and normally lasts between 10 and 15 minutes.

A few days after this, you will carry out a familiarisation trial. This trial will be identical to the main experiment (with the exception that there will be no blood taken) to allow you to familiarise yourself with the procedures and requirements.

The main experiment involves the last four visits to the laboratory; each will be separated by at least one week. During each visit we will ask you to provide a urine sample and then weigh yourself nude in a separate, private room. We will then ask you to position a rectal thermometer, in private, in order to monitor core temperature. A Polar heart rate monitor and skin temperature probes will also be positioned. You will then be asked to consume a fruit-flavoured drink containing either an amount of tyrosine or a flavoured placebo containing no tyrosine. Tyrosine is a naturally occurring amino acid, it poses no health risk and it has been used in several previous studies with no ill-effects. After drinking the solution, you will be required to sit quietly for 1 hour before consuming a second drink, containing the remainder of the tyrosine dose, or the placebo. You will then be required to exercise on a cycle ergometer at a moderate intensity for 60 minutes, in a warm (30°C, 60% relative humidity) environment. Following completion of this you will complete a simulated time trial, where you will be asked to reach an individualised work target as quickly as possible. We will measure how hard you feel you are working and how hot you feel, every 5 minutes throughout the exercise using simple visual scales. Heart rate and core and skin temperature will also be measured every 5 minutes. After the exercise, you will give a second urine sample and weigh yourself nude in private. You will be free to use the shower and changing facilities in the Department before you leave, if you wish. Please allow approximately 3-4 hours for these visits.

Four blood samples, around 10 ml each, will be collected from a forearm vein during each visit by a trained individual. One immediately before you drink the first half of the tyrosine or placebo solution, one immediately before you start exercising, one sample after the 60 minutes of exercise and a final sample at the end of exercise. The volume of blood collected will therefore be small, about 40 ml in total (3 tablespoons).

It is important to maintain similar conditions during each trial. We will therefore ask you to refrain from unaccustomed strenuous activity, and avoid drinking alcohol for 24 hours prior to each visit. You will be instructed to complete a 24 hour food and exercise diary prior to the first visit and you should consume foods of similar quantity and nature before the second visits.

### **Can I change my mind if I decide I no longer want to take part in the study?**

You are free to leave the study at any time, without giving prior notification or a reason for doing so.

### **Other information**

Should you have any further questions regarding participation in the study, I will be pleased to answer them, and may be contacted on:

Nicholas Gregory, email, [nig5@aber.ac.uk](mailto:nig5@aber.ac.uk), tel: 07825538040



**If you have any complaints about any aspect of this study you can contact:**

Academic Supervisor: Dr Les Tumilty, email: llt07@aber.ac.uk, tel: 01970628425

Institute Director of Research: Prof Jamie Newbold, email: cjn@aber.ac.uk, tel:  
01970622242

**SPORT AND EXERCISE SCIENCE**

**INFORMED CONSENT**

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***The effect of varied tyrosine doses on exercise performance in the heat***

**NAME OF VOLUNTEER:** .....

**PRINCIPAL INVESTIGATOR:** Nick Gregory

**ADDITIONAL INVESTIGATORS:** Dr Les Tumilty, Dr Rhys Thatcher

I have read the volunteer information sheet on the above study and have had the opportunity to discuss the details and ask questions. The investigator has explained to me the nature and purpose of the tests to be undertaken. I understand fully what is proposed to be done.

☐

I have agreed to take part in the study as it has been outlined to me, but I understand that I am completely free to withdraw from the study at any time I wish.

☐

I understand that these trials are part of a research project designed to promote scientific knowledge, which has been approved by the Institute of Biological Environmental and Rural Sciences Ethics Committee, and may be of no benefit to me personally.

☐

I understand that participation in the research project involves maximal intensity exercise on a number of occasions

☐

I understand that participation in the research study involves multiple blood samples being taken

☐

I understand any data collected will be kept confidential, and only be seen by principal investigator and supervisor.

☐

I hereby fully and freely consent to participate in the study which has been fully explained to me.

☐

**SIGNATURE OF VOLUNTEER:** .....

**Date:** .....

I confirm that I have explained to the volunteer named above, the nature and purpose of the tests to be undertaken.

**SIGNATURE OF INVESTIGATOR:** .....

**Date :** .....

# **Participate in an exercise study and receive a free fitness test!**

## Want to find out how fit you are?

The Institute Biological, Environmental and Rural Sciences at Aberystwyth University are looking for men who are fit and healthy, aged 18 – 45 and who exercise regularly to take part in a study. We will be exploring a potential performance enhancing nutritional supplement during cycling in the heat




## What do you get for participating?

We will give you an accurate measurement of your aerobic fitness level ( $\dot{V}O_2\text{max}$ ) as well as the opportunity to ask advice on sports nutrition and training.



## Contact

For further information please contact Nick Gregory by email at [nig5@aber.ac.uk](mailto:nig5@aber.ac.uk)

	<p style="text-align: center;"><b>Aberystwyth University Ethics Committee for Research Procedures</b></p> <p style="text-align: center;"><b>ECRP06: Postgraduate Research Assessment Form</b></p>
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For all PhD and Master's research involving human subjects or human tissue.

Section A: To be completed by the Postgraduate Student

**Proposed Research Title:** The effect of different dosages of tyrosine on exercise performance in the heat

**Student Name:** Nicholas Gregory

**Supervisor(s):** Dr Les Tumilty, Dr Rhys Thatcher

**Source of Funding:** Self-funded

**Start of MPhil Registration:** September 2014

**Please outline briefly the focus and purpose of your proposed research:** There is a large body of evidence which demonstrates that exercise performance is impaired in hot and humid conditions compared to cooler conditions. A number of studies have assessed the effectiveness of tyrosine supplementation on the ability to complete a prolonged period of exercise in a hot condition. Tyrosine is a major physiological amino acid precursor to brain dopamine. It is suggested that a high ratio of brain dopamine extends prolonged exercise while conversely stating that when dopaminergic activity is reduced, fatigue is precipitated by a loss of motivation and arousal (Davis and Bailey, 1997), particularly during prolonged exercise in the heat (Watson et al., 2005).

Tumilty et al. (2011) demonstrated that acute supplementation of tyrosine (150 mg kg body mass<sup>-1</sup>) was associated with increased endurance capacity in the heat. However, a separate study by Tumilty, Davison, Beckmann and Thatcher (2014) indicated that the same acute dose of tyrosine failed to influence endurance performance in the heat. These conflicting results could be due to the adoption of an insufficient tyrosine dose under the more physiologically demanding conditions of a performance trial, compared to a capacity trial. No study has examined the effect of different tyrosine doses on exercise performance in the heat. Therefore, the purpose of this investigation is to administer different doses of tyrosine to ascertain which dose, if any, enhances

exercise performance in the heat. It is intended that the results of the study will assist in identifying the effectiveness of tyrosine supplementation, specifically highlighting what the optimum dose is. The study will also advance current understanding and knowledge in this field and will provide further insight in to the mechanisms which limit exercise and/or work tolerance under extreme environmental conditions.

**Please outline briefly the methods that you propose to employ in the research:** 10 participants will visit the laboratory on 6 separate occasions. The first two visits will consist of a ramp test, to establish maximal oxygen uptake, followed by a familiarisation trial, to accustom subjects to future trials and appease any concerns they may have. These will be separated by at least 48 hours. The following four visits will be experimental trials and will be separated by at least 7 days. The experimental trials will consist of a placebo trial in which a sugar-free drink with microcrystalline cellulose (a calorie-free food constituent) will be administered, and three trials in which the same sugar-free drink will be administered, with one of three different doses of tyrosine (150, 300, 400 mg kg body mass<sup>-1</sup> tyrosine). The order of these trials will be completed in a randomised, crossover fashion and the tyrosine and placebo drinks will be administered in a double-blind fashion. On arrival at each experimental trial, subjects will be required to provide a blood sample (acquired by a standard venepuncture method) before being given a drink containing 300 ml of sugar-free fruit flavoured squash and ordinary tap water with either half the total dose of tyrosine or a placebo (same drink volume with a small amount of microcrystalline cellulose). Following ingestion of the drink, subjects will be required to sit quietly for one hour, provide a second blood sample, before consuming a second 300 ml drink containing either the remainder of tyrosine or placebo dose. Subjects will then cycle for 60 minutes in the heat at 10% Δ, which is a moderate intensity and will be well within the capabilities of each participant. At the end of the 60 minute period of cycling, participants will provide a third blood sample before completing a simulated time trial, requiring completion of an individualised target work quantity, lasting approximately 20 – 25 minutes. Following completion of the time trial, participants will provide a fourth blood sample. All exercise will be completed within the environment chamber at a temperature of 30°C and 60% relative humidity. Heart rate, core temperature, skin temperature, RPE and thermal sensation values will be recorded throughout each experimental trial. Core temperature will be monitored every 5 minutes throughout the cycle exercise. Skin temperature at four sites (via skin thermistors) will also be recorded every five minutes throughout the rest and cycle exercise periods, and recorded using an electronic data logger. Heart rate will be recorded every five minutes throughout the resting and exercise periods using radiotelemetry. Ratings of perceived exertion will be assessed every five minutes throughout exercise using a validated visual analogue scale (Borg, 1982). Thermal sensation will also be assessed after every five minutes of exercise using a separate visual analogue scale (Parsons, 2002).

Please complete the following checklist:

Does the research involve participants under the age of 18?	<b>YES</b>	NO	<b>N/A</b>	If yes, refer to ECRP
If yes, is the involvement of children central to the research?	<b>YES</b>	<b>NO</b>	N/A	If yes, please justify <sup>1</sup>

<sup>1</sup> Where research can be confined to adults without detrimental effect, children should not be involved. If the investigators feel that children are essential to the research, this should be justified in the research proposal submitted to the ECRP.

If children are involved, will access to participants be obtained through appropriate and responsible gatekeepers?	YES	NO	N/A	
If yes, will access to participants be obtained through appropriate and responsible gatekeepers?	YES	NO	N/A	
Will consent be sought from both children and parents?	YES	NO	N/A	
Does the research involve other vulnerable participants?	YES	NO	N/A	If yes, refer to ECRP
If yes, is the involvement of vulnerable participants central to the research?	YES	NO	N/A	If yes, please justify <sup>2</sup>
Does the research involve students as participants?	YES	NO	N/A	If yes, refer to Head of Department/ Director of Research
If yes, is the involvement of students central to the research?	YES	NO	N/A	If yes, please justify <sup>3</sup>
Does the proposal involve covert research?	YES	NO	N/A	If yes, refer to ECRP
If yes, are you confident that data cannot be obtained through non-covert methods?	YES	NO	N/A	
Will the research participants be fully informed about the purpose of the research?	YES	NO	N/A	See attached participant information sheet
Will the informed consent of the research participants be obtained?	YES	NO	N/A	See attached informed consent agreement
Will anonymity be extended to all research participants?	YES	NO	N/A	
If not, will participants be informed of this?	YES	NO	N/A	
Is it clear to participants that they can withdraw at any time?	YES	NO	N/A	
Is it clear to participants that their data does not enjoy	YES	NO	N/A	

<sup>2</sup> Where research can be confined to non-vulnerable participants without detrimental effect, vulnerable participants should not be involved. If the investigators feel that vulnerable participants are essential to the research, this should be justified in the research proposal submitted to the ECRP.

<sup>3</sup> Where research can be confined to non students without detrimental effect, students should not be involved. If the investigators feel that students are essential to the research, this should be justified in the research proposal submitted to the Head of Department/Director of Research.

legal privilege?

Have arrangements been made to ensure that guarantees of confidentiality and anonymity can be honoured?	YES	NO	N/A	See ECRP7 form
If data is to be shared with other researchers in the future, will the consent of participants be sought?	YES	NO	N/A	
Will appropriate measures be put in place for the secure and confidential storage of data?	YES	NO	N/A	See ECRP7 form
In the case of elite-level interviews, will consent be sought for the attribution of quotes and data?	YES	NO	N/A	
Will the research expose participants to physical or psychological conditions different to those experienced in everyday life?	YES	NO	N/A	See ECRP7 form
Have measures been put in place to minimise or alleviate any distress which the research may cause?	YES	NO	N/A	See ECRP7 form
Does the proposal involve research via the internet?	YES	NO	N/A	Please provide details
Does the proposal involve interviews or questionnaires?	YES	NO	N/A	
In the case of fieldwork, has a risk assessment been produced?	YES	NO	N/A	
In the case of action research, evaluation and consultancy, are measures in place to ensure balanced participation?	YES	NO	N/A	Please provide details
In the case of externally funded research, have the respective obligations of the researcher and funding body been clarified?	YES	NO	N/A	Please provide details
Have the rights of researchers to disclose the aims and background of the project, and to publish and disseminate results been clarified?	YES	NO	N/A	See ECRP7 form
Are the resources adequate for completion of the project?	YES	NO	N/A	If no, project should not be undertaken
Will participants be consulted prior to publication?	YES	NO	N/A	
Will final project reports be made available to participants?	YES	NO	N/A	

Do you believe that the proposed research complies with AU's Template for Research Involving Human Tissue or Participants?      YES      **NO**      **N/A**      If no, please explain below

Student's \_\_\_\_\_ signature:

Signature \_\_\_\_\_ of \_\_\_\_\_ Supervisor(s):

Date: \_\_\_\_\_

### Section B: To be completed by the Director of Research/Director of Postgraduate Studies

This checklist is designed to assist the Director of Research/Director of Postgraduate Studies in the process of deciding whether a proposal satisfies AU's Template for Research Involving Human Tissue or Participants. If the answer to any of the questions is negative or doubtful, the project should be sent back to the Principal Investigator or referred to the **University Ethics Committee for Research Procedures**. If in any doubt please contact the Secretary of the Committee in the Deans' Office.

Is the research proposal of good design?	YES	NO	N/A	
Are arrangements for the supervision of the project appropriate?	YES	NO	N/A	
Is the research carried out or supervised by competent researchers?	YES	NO	N/A	
Do the foreseeable benefits of the research outweigh the foreseeable risks?	YES	NO	N/A	
Does the proposed research pose only minimal and predictable risk to the researcher?	YES	NO	N/A	If no, refer to ECRP
Does the proposed research pose only minimal and predictable risk to the research subject?	YES	NO	N/A	If no, refer to ECRP
Does the research proposal include sufficient and appropriate procedures to obtain the informed consent of the research subjects?	YES	NO	N/A	
Is it clear to the subjects that they may withdraw at any time?	YES	NO	N/A	
Have arrangements been made to ensure that material obtained from or about a subject remains confidential?	YES	NO	N/A	
Where the proposal involves interviews and/or questionnaires, are measures in place to monitor interview structures and/questionnaire design?	YES	NO	N/A	
If the proposal involves covert research, is sufficient justification provided?	YES	NO	N/A	
If the proposal involves subjects aged under 18, is sufficient	YES	NO	N/A	



justification provided?

If the proposal involves vulnerable participants, is sufficient justification provided? YES NO N/A

justification provided?

Does the research proposal comply with the Template for Research Involving Human Tissue or Participants? YES NO N/A If no, please explain below

(For research proposals to be referred to the **University Ethics Committee for Research Procedures**) Have forms ECRP01 and ECRP02 been completed? YES NO N/A

The above-named proposal merits a Level \_\_\_\_ grading, according to the Statement for Research Councils.

Signed:



Date: 04.12.14\_\_\_\_\_

If the proposed research is judged to comply with the University's Template for Research Involving Human Tissue or Participants, indicate whether you feel it to be level one or two, and retain a copy of this form for the Department's report to the University's Ethics Committee for Research Procedures.

If the proposal involves covert research, subjects under the age of 18, vulnerable participants, or exposes participants to any physical or psychological conditions different to those experienced in everyday life and does not incorporate steps to control or minimise these conditions, and/or the researcher has not demonstrated the necessary skills and understanding, and/or the project does not fulfil all legal and university regulations, indicate which of the levels detailed in the Statement for Research Council you consider it to merit and submit further details of the project on Forms ECRP01 and ECRP02 to the Secretary of the University Ethics Committee for Research Procedures in the Deans' Office, so that the proposal may be further considered by the Committee.



**Aberystwyth University Ethics Committee for Research Procedures**

**Application for Approval of Experiment and Investigation Involving Human Subjects**

This form does not need to be submitted for proposals which have met the criteria set under the generic protocols approved by each department, and are reported annually to the University Ethics Committee for Research Procedures.

**This form is to be used in conjunction with form ECRP02.** Please complete both forms and return to

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### ***Section A: Brief details of the Application***

**1. Title:** The effect of varied dosages of tyrosine on exercise performance in the heat

**2. Name of Principal Investigator(s): [attach a statement of their qualifications and prior experience, which are relevant to the proposed project]**

Title: Mr      Forename: Nick      Surname: Gregory      Qualification: BSc

Research student leading to an MPhil qualification, IBERS, Aberystwyth University, Aberystwyth  
Previous experience with similar protocols and has used the equipment throughout his undergraduate degree

E-mail: nig5@aber.ac.uk

Title: Dr      Forename: Les      Surname: Tumilty      Qualification: PhD.

Personal experience of the proposed protocol, and several years of experience with similar procedures (please see attached statement)

E-mail: llt07@aber.ac.uk

**3. Department(s) involved:**

IBERS, Aberystwyth University, Aberystwyth.

**4. Names and status of any other members of staff involved:**

Title: Dr      Forename: Rhys      Surname: Thatcher      Qualification: PhD

Personal experience of the proposed protocol, and several years of experience with similar procedures (see attached statement)

E-mail: ryt@aber.ac.uk

**5. Source of financial support (if any) for proposed investigation:**

None.

**6. Will any part of the investigation be carried out under the auspices of an outside organisation? If so, give details, including names and status of co-investigators:**

No.

**7. Will students be involved in carrying out the investigation?**

Yes, the research will be carried out as partial requirement of an MPhil qualification.

## **Section B: Abstract of Project (Continue on a separate sheet if necessary)**

### **1. What is the purpose of the investigation? Is it intended to benefit the subject?**

There is clear evidence that exercise is impaired in hot and humid environments compared to cooler climates (Tattersson, Hahn, Martin and Febbraio, 2000; Gonzalez-Alonso, Teller, Andersen, Jensen, Hyldig and Nielsen, 1999; Galloway and Maughan, 1997). The impairment of exercise in the heat has been associated with increased central fatigue which is defined by Nybo (2010) as an impaired ability to sustain muscle activation and increased subjective effort during exercise. It is suggested by Davis and Bailey (1997) that altered brain neurotransmitter activity may account for this fatigue. Specifically, a high ratio of brain dopamine to brain serotonin is suggested to sustain motivation and arousal during prolonged exercise and a low ratio induces central fatigue. This is particularly relevant to prolonged exercise in the heat as the maintenance of brain dopamine activity appears to specifically influence the ability to continue exercise (Watson et al., 2005). The amino acid tyrosine is a major nutritional precursor to brain dopamine (Glaeser, Melamed, Growdon and Wurtman, 1979). Tumilty et al. (2011) demonstrated that acute supplementation of tyrosine ( $150 \text{ mg kg body mass}^{-1}$ ) was associated with increased endurance capacity in the heat. However, a separate study by Tumilty, Davison, Beckmann and Thatcher (2014) indicated that the same acute dose of tyrosine failed to influence endurance performance in the heat. These conflicting results could be due to the adoption of an insufficient tyrosine dose under the more physiologically demanding conditions of a performance trial, compared to a capacity trial. No study has examined the effect of different tyrosine doses on exercise performance in the heat. Therefore, the purpose of this investigation is to administer different doses of tyrosine to ascertain which dose, if any, enhances exercise performance in the heat.

The exercise protocol adopted is identical to one used previously in our laboratory (Tumilty et al. 2014). It is intended that the results of the study will assist in identifying the effectiveness of tyrosine supplementation, specifically highlighting what the optimum dose is. The study will also advance current understanding and knowledge in this field and will provide further insight in to the mechanisms which limit exercise and/or work tolerance under extreme environmental conditions. The results will have potential application to several areas in which physical work is performed in warm/hot environments such as armed and uniformed services, sport and manual labour intensive employment.

Direct benefit to the participants will include the use of exercise equipment and expertise within the sport and exercise science building, including fitness testing which will enable the identification of an individual's aerobic fitness ( $\dot{V}O_2$  peak). Also, as some participants will be drawn from the student population, they will gain a valuable first hand insight into the research process.

### **2. Will invasive procedures (medical or surgical) be used?**

Blood samples will be taken from an arm vein (by a standard venepuncture method) during each visit by suitably trained staff: one before participants are given the supplement, another sample one hour after drink ingestion, immediately before they start exercising, one sample after cycling for 60 min, and a final sample when they stop exercising at the end of the time trial. The total

amount of blood sampled during each visit will be minimal, approximately 40 ml, or 3 tablespoons.

A rectal thermistor, positioned 10 cm beyond the anal sphincter will be used to record core temperature, as per RA SES9. This is a common method of core temperature measurement in these types of investigations which poses no health risk to the participant. The thermistor is thoroughly sterilised following each use, and sealed in a protective bag between visits, as per SOP SES5.

**3. In the course of the investigation, might pain, discomfort (including psychological discomfort), inconvenience or danger be caused?**

Blood sampling carries a small risk of bruising or infection, but only trained staff obtain samples and with good practice, any risk is minimal.

Exercising in a warm environment can cause dizziness or light headedness in some individuals, however with constant monitoring of core temperature as well as staff being vigilant at all times, the risk is minimal. If participants feel unwell at any time they are free to stop exercising should they wish to do so. Also, the proposed participant population will be familiar with the discomfort associated with strenuous exercise.

The use of the tyrosine drink poses no health risk and has been used in several previous studies with no ill-effects (Shurtleff et al., 1994; Strüder et al., 1998; Chinevere et al., 2002; Mahoney et al., 2007; Tumilty et al., 2011; Tumilty et al., 2014).

**4. Give details of the proposed research protocol including equipment to be used and any safeguards or precautions to be taken. Include a statement of the findings of any risk analysis undertaken with regard to the subject's safety and well being.**

Participants will initially complete an incremental exercise test on a cycle ergometer to measure their peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ), using a computer analysis system for breath-by-breath analysis. The incremental exercise test will involve subjects breathing through a mouthpiece, which will be sterilised between each visit (this procedure is covered under the Department's generic physiology procedures and risk assessments).

Following this, participants will complete a familiarisation visit to accustomize themselves with the procedures and environmental conditions as well as the sampling protocol. This will be identical to the placebo trial, including administration of the placebo drink. Finally, four main visits will be carried out which will be similar in every respect to the familiarisation visits, except in three of these trials, tyrosine will be administered in three different doses, as opposed to the placebo drink.

Each of these visits will require participants to arrive at the lab in the morning after an overnight fast of at least 8 hours. Nude body mass will be assessed and a urine sample will be taken in order that urine osmolality can be measured (to ensure that they are hydrated prior to beginning exercise). If participants are hypohydrated (urine osmolality > 700 milliosmoles per kg; ACSM, 2007), then testing will be rescheduled. A blood sample will be taken from an arm vein by a standard venepuncture method. Participants will then be required to ingest one of four drinks in a randomised, double-blind fashion: 300 ml of lemon and lime flavoured sugar-free squash and

ordinary tap water with the addition of either half of the prescribed experimental dose (150, 300 or 400 mg per kg body mass tyrosine), or the same volume of fluid containing a small amount of microcrystalline cellulose (as placebo). Following ingestion, participants will be required to sit quietly for one hour, after which they will consume the remaining half of the prescribed experimental tyrosine dose or the placebo in a further 300 ml of sugar-free squash and ordinary tap water. They will give a second blood sample, before entering the heat chamber (30°C and 60% relative humidity) to commence exercise. The initial bout of exercise will require participants to exercise for 60 minutes at a power output equivalent to 10%  $\Delta$  (power outputs are calculated individually for each participant with data derived from the previous ramp test). This is a moderate intensity and is well within the abilities of healthy individuals. The 60 minutes of cycling will then immediately be followed by a third blood sample before a self-paced cycling time trial is completed where participants will be required to complete a set amount of work as quickly as possible. The set amount of work will be individualised to each participant, calculated based upon the data from the initial ramp test, and will last approximately 20 to 25 minutes. A fourth blood sample will be taken upon completion of the time trial.

Core temperature will be monitored every 5 minutes throughout the cycle exercise in accordance with the departmental risk assessment for exercise in the environmental chamber. Skin temperature at four sites (via skin thermistors) will also be recorded every five minutes throughout the rest and cycle exercise periods, and recorded using an electronic data logger.

Heart rate will be recorded every five minutes throughout the resting and exercise periods using radiotelemetry.

Ratings of perceived exertion will be assessed every five minutes throughout exercise using a validated visual analogue scale (Borg, 1982). Thermal sensation will also be assessed after every five minutes of exercise using a separate visual analogue scale (Parsons, 2002).

Standard statistical methods will be used to analyse data. For example, a repeated measures 2-way ANOVA (time  $\times$  trial) will be used to analyse differences between the placebo and experimental conditions. Time to complete the time trial will be analysed using a 1-way repeated measures ANOVA. Data will be analysed at a significance level of 0.05.

## **5. What will be the duration and frequency of the procedures?**

At least 48 hours will separate the initial incremental cycle test and the first familiarisation visit, and at least seven days will separate each of the familiarisation and main visits. The incremental test will be completed in around 30- 45 minutes, and the familiarisation and main visits will require approximately 3-4 hours for each visit.

## **6. What steps will be taken to safeguard against the effects of any repetitive research?**

Adequate rest will be given to ensure complete recovery between visits. Also, participants will refrain from unaccustomed strenuous activity for 24 hours before each visit. Participant numbers are small so it is unlikely that repetitive research will take place within the study. A secure database will be held (along with screening questionnaires- in accordance with departmental Health and Safety procedures) within the department to prevent repetitive research between studies.

**7. Does this research break any laws?**

No.

**8. Do you have any issues relating to this project that you would like to discuss with the Committee?**

No.

**9. Any further relevant information.**

None.

**Section C: Information on Subjects**

NB: No subject should be admitted to a trial before the University Ethics Committee for Research Procedures has issued its written approval.

**1. How many subjects will be involved?**

10 participants will be adequate to highlight any effect of the proposed drink on exercise performance. A previous study in our laboratory using 8 subjects was adequate to identify an increase in exercise time to exhaustion in the heat at a constant-load, submaximal intensity (i.e. exercise capacity as opposed to performance), between a tyrosine-containing drink (150 mg kg body mass<sup>-1</sup>) and placebo drink containing no tyrosine, with an effect size of 0.63 and a resultant statistical power value of 0.78.

**2. What is the age and sex of each?**

It is envisaged that the majority of participants will be physically active males recruited from the student and staff populations of Aberystwyth University, as well as local sporting clubs. The age range of participants will be 18 – 45. Because of the physically demanding nature of the study, anyone with a history or predisposition to musculoskeletal, or cardiorespiratory, or metabolic disease will be excluded.

**3. How are the subjects to be recruited? Provide**

- (i) a copy of the advertisement/letter of invitation to be used and**
- (ii) a copy of the information sheet<sup>4</sup> given to subjects.**

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<sup>4</sup> The information sheet and the informed consent may include similar text. The distinction is that the information sheet is something the participant can take away. The informed consent is something the individual signs and is kept by the researcher.

Subjects will be recruited by means of posters around the University campus and by weekly e-mail bulletin distributed by the University Information Services and also via the sports and activities officer within Aberystwyth University.

**4. How and when will their consent be obtained? Provide a copy of the informed consent form to be used.<sup>5</sup>**

Informed consent will be obtained following an initial meeting with potential participants, during which all potential risks and discomforts associated with the study will be outlined. Verbal and written informed consent will be obtained from all participants before any testing takes place in line with the departmental generic ethics document.

**5. How will the subject's physical and mental suitability for participation be assessed?**

A health questionnaire will be completed by all potential participants at the initial meeting with investigators, and also during each visit to the laboratory, before exercise commences, to assess subject's physical and mental suitability.

**6. Will the subject's doctor be notified? If so, please provide sample letter to subject's GP.**

No.

**7. What steps, if any, will be taken to safeguard the confidentiality of the results of the investigation?**

Participants will be assigned a code on commencing the study, and will only be identifiable by name to the study investigators. All collected data will be stored securely within the department on password protected PCs, and will not be shared with outside parties without prior consent from the individual concerned.

**8. What do you consider are the major ethical issues in this proposal?**

None

**9. Any further relevant information.**

None.

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<sup>5</sup> This must detail the basic purpose of the study, a fair explanation of what the participant will do, the risks and benefits, and a statement that the participant may withdraw at any time without prejudice. Since participants may commonly be students, where appropriate there should be a statement that involvement in or withdrawal from the project will have no implications to their academic standing within the university.



## Section D: Declaration

The following declaration should be signed by the Principal Investigator(s) and the relevant Head of Department.

I certify that to the best of my knowledge the information given above, together with any accompanying information, is complete and correct and I approve the application for submission to the University Ethics Committee for Research Procedures.

**Principal  
Investigator(s)**

**Date**

\_\_\_\_\_

\_\_\_\_\_

**Head of Department**

**Date**

\_\_\_\_\_

\_\_\_\_\_

Encs: (tick box to indicate that relevant document is attached)

- (i) Statement of Principal Investigator(s) relevant qualifications and prior experience
- (ii) Proposed advertisement/invitation to recruit subjects
- (iii) Proposed information sheet for subjects
- (iv) Proposed informed consent form
- (v) Proposed sample letter to subject's GP
- (vi) Form ECRP02


ECRP7 proforma form01/KRB February 2003

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